Comparison of Vaccine and Wild-Type Measles Virus strains effect on Polymorphonuclear Leukocyte function : an *in vitro* analysis

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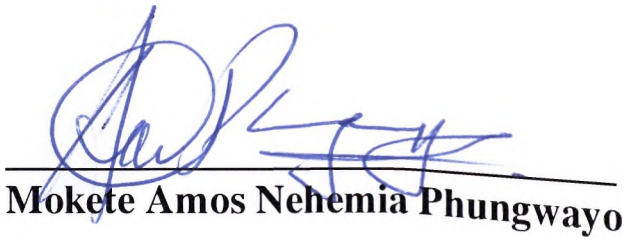
DECLARATION

I hereby declare that this dissertation is my own, unaided work. It is being submitted to the Faculty of Health Sciences of the University of the Witwatersrand, Johannesburg, for the degree of Master of Science in Medicine. This work has not been submitted before for any degree or examination at this or any University.



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ABSTRACT

Measles virus (MV)-infected persons have been found to be at a greatly increased risk of developing respiratory infections caused mainly by *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Secondary microbial infections have been implicated as important causes of morbidity and mortality in patients with measles. Polymorphonuclear leukocytes (PMNL), the first cells of the immune system to migrate to the site of microbial invasion, are the principal cells involved in the immune response to invasion of the host by microbes, particularly bacteria and fungi.

This study set out to determine whether MV has any directs effects on PMNL functions, that could account for the increased susceptibility of MV-infected persons to secondary microbial infections. Therefore, the specific objectives of this study were :

(a) to determine which, if any, PMNL functions (i.e. chemotaxis, degranulation, phagocytosis, oxidative burst), are altered by exposure to MV *in vitro*.

(b) to determine whether MV can, directly or indirectly, induce apoptosis *in vitro*.

(c) to determine whether virus attenuation or heat inactivation of MV strains have any effect on these functions.

(d) to determine whether ligation of the MV-specific receptor, CD46, by CD46 antibody can alter any of the PMNL functions mentioned above, or induce apoptosis.

PMNL isolated from 5 to 7 healthy individuals using Ficoll Histopaque®-1077, purified by lysing contaminated red blood cells with ammonium chloride and washed with PBS, were exposed to three MV strains :- Schwartz (vaccine), Jhb 25 (vaccine-like) and D1317 (wild-type), and their heat-inactivated forms (inactivated by heating at 56^0 C for 1 hour). Each of these strains represent different degrees of MV attenuation : vaccine > vaccine-like > wild type, and all assays used similar virus inputs. PMNL assays and an apoptosis assay were conducted on the above-mentioned samples.

A specific antibody to CD46, was used to ligate CD46 receptors on PMNL, and its ability to affect interleukin-8 (IL-8)-induced chemotaxis, and to induce apoptosis was determined. An

isotope control antibody, IgG_1 was used in addition to the CD46 antibody. These assays were conducted to determine if by ligating the CD46 receptor used for entry by MV, similar effects on PMNL could be noted to those of the MV strains.

Chemotaxis was significantly reduced by exposure to all MV strains, the greatest reduction being seen in the wild-type D1317-exposed PMNL. A slight recovery of function was observed when PMNL were exposed to heat-inactivated forms of these strains. However, this recovery was not significant, as chemotactic indices obtained remained significantly decreased relative to the uninfected control.

A reduction in phagocytic ability was observed in PMNL exposed to the three MV strains. Both the proportions of cells phagocytosing bacteria (*E. coli*), and the number of bacteria within the cells (measured as fluorescence intensity), were reduced relative to the uninfected control. The reduction was more pronounced in the wild-type D1317-exposed PMNL. Heat inactivation of viral strains brought about some improvement, but failed to restore the PMNL function. It was clear that the ability of PMNL to engulf bacteria was compromised by exposure of these cells to the different MV strains.

The trend observed in phagocytosis, was also observed with the oxidative burst function. Both the percentage of cells producing reactive oxygen intermediates, and their mean fluorescence intensity were decreased in PMNL exposed to all three MV strains. Heat inactivation had little impact on the percentage of cells releasing reactive oxygen intermediates.

With degranulation, MV-exposed PMNL showed no changes in the percentages of enzyme (β-glucuronidase) released relative to the uninfected control, in either the absence or presence of IL-8. Inter-strain comparisons also did not show significant differences in the percentages of enzyme released. Furthermore, heat inactivation of all strains had no impact on degranulation of PMNL.

All three MV strains used induced apoptosis of PMNL, the greatest reduction occurring in those exposed to wild-type D1317 MV strain. This strain caused a 90% increase in apoptosis when compared to the uninfected control. Heat inactivation only significantly decreased apoptosis

induced by the wild type MV strain. Even though apoptosis was decreased to some extent by heat inactivation of the MV strains relative to their untreated forms, it was still significantly high when compared to the uninfected control.

Reductions in chemotactic indices were also observed in PMNL exposed to two CD46 antibody concentrations (5 ug/ml and 25 ug/ml), with the greatest reduction observed in PMNL exposed to the higher concentration of the CD46 antibody. Interestingly, exposure to the two concentrations of the isotype control antibody IgG_1 (5 ug/ml and 25 ug/ml) was accompanied by an increase in the chemotactic index, suggesting that ligation of F_c receptors, which bind IgG_1 molecules, may be involved in enhancing chemotaxis. Ligation of the MV cellular receptor, CD46 with a specific antibody, also induced apoptosis. Apoptosis in the isotype control IgG_1-exposed PMNL, although raised relative to the uninfected control, was less than that observed in the CD46-exposed PMNL. Increasing the concentration of IgG_1 antibody led to a reduction in apoptosis. This observation, like the one noted above in chemotaxis, suggests a protective effect here imparted by higher concentrations of the isotype control antibody, IgG_1. Therefore the mechanism(s) involved in the suppression of IL-8 induced chemotaxis and in the induction of apoptosis appears to involve the binding of MV to its cellular receptor on PMNL. This is further supported by the fact that (i) MV does not replicate in PMNL and (ii) exposure of MV to PMNL in all experiments was only for one hour.

In summary, the results indicate that MV has the ability to directly incapacitate a number of PMNL functions (chemotaxis, phagocytosis and oxidative burst), and to induce apoptosis. This most likely occurs at the stage of virus attachment. It is therefore likely that MV, by binding to PMNL *in vivo*, (in particular, the wild-type strain of MV) may play an important role in compromising innate cellular immunity in the MV-infected host, and in so doing predisposes the individual to secondary microbial infections.

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INTRODUCTION

MEASLES VIRUS

Measles virus (MV), the cause of measles, is the most contagious human agent, resulting in frequent acute admissions to paediatric wards in many countries, particularly in third world countries (Nur *et al.*, 1999). The virus belongs to the genus Morbillivirus, within the Paramyxoviridae family of RNA viruses (Örvell, 1994). Despite the availability of a vaccine and the establishment of the global measles vaccination program by the World Health Organization (WHO), approximately one million children die annually from measles, the majority of whom are from underdeveloped countries, where large communities remain unvaccinated and malnourished (Gellin and Katz, 1994a).

In these countries, it is estimated that between three and fifteen percent of children contracting measles die from this disease. In fact, it has been shown that measles and its complications account for more deaths of children worldwide, than do all other childhood vaccine-preventable diseases combined, or than any other single infectious agent (Gellin and Katz, 1994a).

1.1 Classification

Measles virus is a member of the family Paramyxoviridae and shows structural and biochemical features associated with these viruses. It lacks the virion-associated enzyme Neuraminidase and has therefore been grouped into a separate genus, the Morbillivirus. Measles is the type species of this group whose other members include : peste des petits ruminants which infects sheep and goats; rinderpest which infects cattle, canine distemper virus (CDV) which infects dogs, and phocine distemper virus (PDV) which infects seals and sea-lions. All these viruses exhibit antigenic similarities, and all produce similar diseases in their host species. Both MV and CDV can persist in the central nervous system (CNS) and produce chronic neurological diseases. It is not known whether the other members of the group can do likewise (Carter and ter Meulen, 1995).

1.2 Morphology

Measles virus, like all other members of the Paramyxoviridae family, is an enveloped virus (Collier and Oxford, 1993). The virion is bounded by a lipid bilayer envelope, derived from the host cell. Embedded in this envelope are virion glycoproteins (Carter and ter Meulen, 1995). When viewed under an electron microscope, measles virions appear roughly spherical, but pleomorphic and have diameters ranging from 100 to 300 nm (Lund *et al.*, 1984; Griffin and Bellini, 1996). Also embedded in the lipid bilayer through their hydrophobic tails and protruding through as surface projections (peplomers) with a length of 9 to 15 nm, are the viral transmembrane haemagglutinin (H) and fusion (F) glycoproteins (Gerald, *et al.* 1986; Carter and ter Meulen, 1995; Griffin and Bellini, 1996; Oxman, 1997). When examined by electron microscopy, the H proteins peplomers appear conical in shape, while the F proteins peplomers are said to be dumbbell-shaped with ends of unequal sizes (Norrby and Oxman, 1990). Both proteins are estimated to be 15-16 nm length, excluding the transmembrane portions, which are estimated to be an additional 7-8 nm (Bellini *et al.*, 1994). The matrix protein (M) lies on the inside of the envelope, and is thought to interact with both the H and F glycoproteins, and with the nucleocapsid to play a role in virion maturation. The primary nucleocapsid (N) protein encases the virion RNA (Lund *et al.*, 1984; Carter and ter Meulen, 1995; Griffin and Bellini, 1996). The nucleocapsid has a helical structure with a diameter of of 21 nm, a central hole of 5 nm and a pitch of 6 nm. Bound to genomic RNA, are the phospho (P) and large (L) proteins. The total length of free intact nucleocapsid is packed within the envelope in the form of a symmetrical coil.

Virions may also contain actin from the cellular cytoskeleton (Tyrrell and Norrby, 1978; Fragraeus *et al.*, 1983), which is involved in the final steps of budding from the plasma membrane (Stallcup *et al.*, 1983; Bohn *et al.*, 1986). See Figure 1.1.

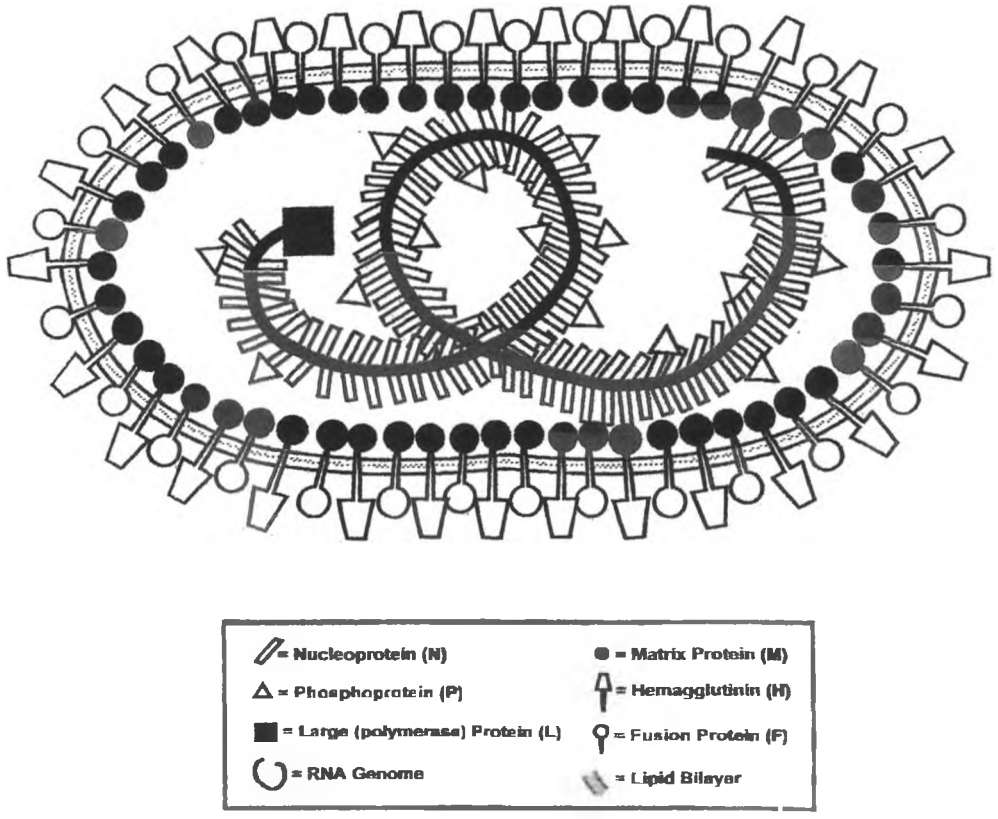


Fig. 1.1 : *Schematic diagram of Measles virus. Adapted from Oxman, 1997.*

1.3 Genome

The genome of MV is made up of a single-stranded, non-segmented RNA of negative polarity. The length of this genome is approximately 16 000 ribonucleotides and its molecular mass approaches 4,5 x 10^6 daltons (Baczko *et al.*, 1983; Lund *et al.*, 1984). It makes up approximately 5% of the nucleocapsid weight (Waters *et al.*, 1972). The entire genome of the Edmonston strain of measles virus has been sequenced (Bellini *et al.*, 1985; Bellini and Englund, 1985; Rozenblatt *et al.*, 1985; Alkhatib and Briedis, 1986; Blumberg *et al.*, 1988). The genome starts from the 3' end with a leader sequence of 55 nucleotides. This sequence shows a high degree of complementarity to 40 nucleotides at the 5' end of the genome, suggesting the possibility for formation of a panhandle structure (Crowley *et al.*, 1988).

The genome encodes six major structural proteins from the six genes and an additional two nonstructural proteins from the P gene. The gene order is as follows : 3' N, P (plus C and V), M, H and L (large polymerase protein) 5' (Richardson *et al.*, 1985; Dowling *et al.*, 1986) with CUU as the intergenic trinucleotide (Crowley *et al.*, 1988; Carter and ter Meulen, 1995). The mRNAs for the MV structural genes are functionally monocystronic with one exception. Within the P gene a second open reading frame is found, encoding for the non-structural protein C. As a third protein, V protein is expressed from the same gene containing the amino-terminal half of P, but with a different, cysteine-rich carboxyl terminal domain (Carter and ter Meulen, 1995).

1.4 The Proteins

The characteristics of the measles virus proteins are summarised in Table 1.1.

1.4.1 Nucleocapsid (N) protein

The N protein is the most abundant of all viral glycoproteins. Its mRNA is the first to be transcribed from the genome. Synthesis of this protein takes place on free ribosomes, folded in the cytoplasm (Gombart *et al.*, 1993), and phosphorylated on serine and threonine residues (Robbins and Bussel, 1979). On polyacrylamide gels, the N protein normally appears as a 60 000 daltons band, but is often cleaved by cellular proteases during extraction to 45 000 and 41 000 daltons products (Graves, 1981). The N protein surrounds viral genomic and messenger RNAs possessing the leader sequence (Castaneda and Wong, 1990) to form the nucleocapsid structures (Udem and Cook, 1984). By so doing, it condenses the RNA into a smaller, more stable and more readily packaged form, and hence confers the helical form on the nucleocapsid giving it a 'herringbone' appearance when viewed under an electron microscope (Carter and ter Meulen, 1995). The function of the N protein is to protect the viral RNA (Örvell, 1994). The nucleocapsid structure is the required template for both replication and transcription, and, in conjunction with the P and L proteins, comprises the ribonucleoprotein complex (Ray and Fujinami, 1987). N can be transported to the nucleus but is usually retained in the cytoplasm by binding to P (Huber *et al.*, 1991). Amino acid sequence conservation is strong in the N-terminal portion of the protein, which contains the P binding site (Gombart *et al.*, 1993).

TABLE 1.1 : Characteristics of the measles virus proteins

Protein	Apparent size		Location		Suggested function/special features
	Amino acid residues	kd	Infected cell	Virion	
Nucleoprotein (N)	525	60	Cytoplasm; nucleus (persistent infections)	Nucleocapsid	Most abundant of viral proteins; closely associated with full-length - and + sense RNA to form nucleocapsids; phosphorylated
Phosphoprotein (P)	507	72	Cytoplasm	Nucleocapsid	More abundant in infected cells than in virions; likely involvement in regulation of transcription and replication; complex formation with N and L; phosphorylated
C	186	21	Cytoplasm and nucleus	Not present	Read from the same transcript as P, but in different reading frames; downregulates transcription ?
V	298	40	Cytoplasm	Not present	Read from an "edited" transcript of P gene; phosphorylated; cysteine-rich COOH terminus; function unknown
Matrix or membrane (M)	335	38	Inner leaflet of plasma membrane cytoplasm (persistent infections)	Inner surface of membrane	Virion assembly and budding; inhibits transcription ?

Fusion (F)	553	60	ER, Golgi, and plasma membrane	Transmembrane (type I) surface conjunction with protein	Glycosylated membrane fusion, virus entry and haemolysis activity in H;18-kd active form is cleaved:F_0 (60 kd)→2 subunits F_1 (41 kd) and F_2
Hemagglutinin (H)	617	78	ER, Golgi, and plasma membrane	Transmembrane (type II) surface protein	Receptor binding; hemagglutination activity; disulfide linked dimer; glycosylated
Large (L)	2,213	200	Cytoplasm	Nucleocapsid	Catalytic component of viral polymerase; least abundant protein; RNA transcription and replication; complex formed with P

Adapted from Griffin and Bellini, 1996

1.4.2 P, C and V proteins

The P protein is one of the three proteins encoded for by the P gene. The other two are the C and V proteins. Only small amounts of the P protein are present in the packaged virion despite the fact that this protein is the most abundant in the infected cell, the reason being its sensitivity to proteolysis. This protein is 72 Kda in size. Although the P and C proteins are translated using different initiator methionine codons and overlapping reading frames, they are encoded from the same messenger RNA (Bellini, *et al.*, 1985). The V protein on the other hand shares the initiator methionine and the amino terminal 231 amino acid residues of the P protein (Griffin and Bellini, 1996). The P protein forms part of the replicase complex (Robbins and Bussel, 1979; Stallcup *et al.*, 1979; Huber *et al.*, 1991). The function of the other two proteins is not clear, although there has been speculation that they are probably involved in regulation of transcription and/or replication (Griffin and Bellini, 1996).

1.4.3 Matrix (M) protein

The M protein is strongly associated with the inner layer of the plasma membrane and the nucleocapsid in infected cells (Hirano *et al.*, 1992). This protein together with the H and F glycoproteins make up the viral envelope (Griffin and Bellini, 1996). M appears to interact with the intracytoplasmic regions of one or both transmembrane glycoproteins (H and F), and the speculation is that by so doing, it stabilizes or organizes the membrane environment in preparation for budding of virions (Tyrrell and Ehrnst, 1979). It also inhibits transcription when bound to the viral ribonucleoprotein (Suryanarayana *et al.*, 1994).

1.4.4 Fusion (F) protein

The F protein and the H protein make up the spikes observed under the electron microscope (Carter and ter Meulen, 1995). Compared to the H protein, the F protein has a reverse orientation. It is anchored in the viral membrane with a hydrophobic stretch of 29 amino acids located close to the C terminus of the molecule (Örvell, 1994). It is synthesised as an inactive protein and needs to be cleaved into two disulfide-linked proteins, a non-glycosylated F1 protein (amino acid

residues 113-550 from the N terminus) with a molecular weight of 41 000 daltons and a glycosylated (three potential glycosylation sites, viz. residues 29, 61 and 67) F2 protein with a molecular weight of about 18 000-20 000 daltons, in order to become biologically active (i.e. exact fusion or haemolytic activity). The N terminus of the F1 protein is formed by a second hydrophobic stretch of 25 amino acids from amino acid residues 113 to 137 with sequence homology to other paramyxoviruses. This hydrophobic terminus is reorientated by cleavage of the F protein, and thus becoming active in membrane fusion. A third hydrophobic domain of 29 amino acids is located in the N terminus of the precursor F protein. This part of the molecule is called the signal peptide and it is used for transportation of F through the rough endoplasmic reticulum. It is cleaved off from the mature F protein. The F protein gives rise to haemolysis inhibiting (HLI) antibodies when injected into laboratory animals. These antibodies can protect against disease caused by measles virus, but, in *in vitro* tests, absent or only low titres of neutralizing antibodies are found in such sera (Örvell, 1994). The F protein plays a role in virus penetration into the host cell and promotes fusion of MV-infected cell membranes (Morgan and Rapp, 1977; Choppin *et al.*, 1981).

1.4.5 Haemagglutinin (H) protein

The H protein is the receptor binding and haemagglutinating (Varsanyi *et al.*, 1984) protein. It is a type II transmembrane glycoprotein that resides on the surfaces of infected cells and virions as a disulfide-linked homodimer (Alkhatib and Briedis, 1986; Schneider-Schaulies *et al.*, 1990). Viewed under the electron microscope, isolated peplomers appear conical in shape (Norrby and Oxman, 1990). This protein which is made up of 617 amino acids, is anchored in the viral membrane by a hydrophobic region, 24 amino acids in length, located close to the N terminus of the molecule. The protein has five potential N-linked glycosylation sites closely located between amino acids residues 168-240 in the N-terminal part of the molecule (Örvell, 1994). The H protein is closely associated with the F protein on the cell surface (Malvoisin and Wild, 1993) and acts in conjunction with F protein during fusion and entry (Wild *et al.*, 1991; Cattaneo and Rose, 1993). It is folded and dimerized in the endoplasmic reticulum (ER) (Bellini *et al.*, 1983; Malvoisin and Wild, 1993), and has 13 strongly conserved cysteines, all in the ectodomain (Griffin and Bellini, 1996). It induces the production of neutralizing antibodies when injected

into laboratory animals. The epitopes giving rise to neutralizing and protective antibodies have not yet been fully defined (Örvell, 1994).

1.4.6 Large (L) protein

This protein is present in small quantities in the infected cell and is associated with the viral nucleocapsid in the cell and in the virion (Stallcup *et al.*, 1979). It has been found to be highly homologous to the polymerases of other negative strand viruses. It contains a duplicated Gly-Asp-Asp-Asp motif flanked with hydrophobic amino acids common to RNA polymerases (Blumberg *et al.*, 1988).

1.5 Cellular receptor

The membrane cofactor CD46 has been identified as the cellular receptor for MV. This cellular receptor is present on human and monkey cells which interact with the H protein, and is a glycoprotein (Norrby, 1962; Krah, 1989). It is the only glycoprotein that has demonstrated its ability to confer MV binding and replication to previously non-susceptible murine cells (Dorig *et al.*, 1993; Naniche *et al.*, 1993). This receptor was identified by the generation of a monoclonal antibody that blocked infection of HeLa cells (Naniche *et al.*, 1992), and was subsequently shown to recognize CD46 (Naniche *et al.*, 1993). This receptor is distributed throughout human tissue and has complement regulatory function. It normally binds complement components C3b and C4b and acts as a cofactor in the proteolytic activation of C3b/C4b by factor I (Liszewski and Atkinson, 1992). MV infection of cells or expression of the H protein alone leads to down-regulation of CD46 by rapid internalization from the cell surface (Naniche *et al.*, 1993). This may affect or facilitate entry. Receptor-mediated endocytosis is probably not required for entry because the virus membrane is able to fuse with the cell membrane at neutral pH (Griffin and Bellini, 1996).

1.6 Replication of the virus

Replication of the measles virus occurs initially in the epithelial cells which line the buccal

mucosa and the respiratory tract (Kempe and Fulginiti, 1965; Moench *et al.*, 1988; Gellini and Katz, 1994b). Following replication in these tissues, it spreads to local lymphatic tissues where amplification occurs in the lymph nodes producing a primary viraemia. This results in the spread of the virus to multiple lymphoid tissues and other organs, including skin, kidney, gastrointestinal tract and liver (Kempe and Fulginiti, 1965; Modlin, 1991). Replication continues in endothelial cells, epithelial cells and monocytes/macrophages in these organs (Moench *et al.*, 1988). The process of replication starts with the attachment of the virion to the cell membrane of the host cell, through a specific affinity between the viral envelope membrane protein, probably haemagglutinin, and a host cell receptor. Fusion of the viral envelope with the cell membrane follows, allowing the viral genome to penetrate the cell. This event is believed to be dependent on cleavage of the viral envelope F protein by a host cell enzyme and subsequent fusion activity by the activated F protein (Choppin *et al.*, 1981; Modlin, 1991). Within a period of 24 hours following the penetration of measles virus genome into the host cells, viral RNA synthesis resumes (Portner and Bussel, 1973; Fournier *et al.*, 1983).

Firstly, the negative stranded measles virus RNA is transcribed into a complimentary (positive) RNA message (Kingsbury, 1974; Modlin, 1991). The N gene is the first to be transcribed as the viral polymerase starts transcription at the 3' end of the virion RNA (Örvell, 1994). A transcription signal (gene-start) made up of 10 nucleotides is found at the 3' end of each of the genes to be transcribed. At the end of each gene, there is a termination sequence usually made up of 13 or 14 nucleotides. About 4 to 7 adenine molecules separates the termination signal from the intergenic sequence. The polymerase normally passes over this intergenic sequence to begin transcription at the next signal (i.e. the intergenic sequence is generally not transcribed by the polymerase). This facilitates the transcription of genes in a linear fashion (Örvell, 1994). From the resultant monocystronic mRNA various viral structural proteins are translated. This takes place on the polyribosomes. Formation of virion RNA also takes place here.

Membrane glycoproteins are then transported to the cell membrane through the endoplasmic reticulum and Golgi membranes. Complete cleavage of the F protein into F1 and F2 is normally achieved after 5 hours. Although a portion of this protein may reach the cell surface uncleaved, uncleaved F is not incorporated into the mature virion (Örvell, 1994). The N can be found within

the nuclei of infected cells, without any other viral structural protein being present. This is unique for measles virus and has not been reported for other paramyxoviruses (Örvell, 1994).

Transport of the M protein to the cell membrane is next. This protein lines the plasma membrane, where it interacts with the cytoplasmic tail of the envelope glycoproteins. One domain of the M protein interacts with the inner side of the cell membrane and another with the nucleocapsid (Örvell, 1994). Closely associated with the nucleocapsids intracellularly and in mature virions, are the actin filaments. These filaments assist in the formation of viral buds at the cell membrane. Normally, the entire process of viral replication is completed approximately 20 hours following infection, and the infectious particles can be detected in the extracellular environment (Örvell, 1994).

1.7 Biological characteristics of MV

1.7.1 Haemagglutination and Haemadsorption

A physiologic pH and a temperature of 37^0 C are required for haemagglutination to occur (Hirano *et al.*, 1992; Takenhara *et al.*, 1992). The virus binds only primate erythrocytes and most efficiently agglutinates red blood cells from the African green monkey (Sheshberadaran et al., 1983). This is consistent with the distribution of the CD46 molecule, which is absent on human but present on primate red blood cells (Liszewski and Atkinson, 1992). Cells infected with MV can be identified by their ability to adsorb monkey erythrocytes (haemadsorption). Both haemagglutination and haemadsorption are properties of the H glycoprotein (Hirano *et al.*, 1992; Takenhara *et al.*, 1992).

1.7.2 Fusion and Syncytia formation

The F glycoprotein is responsible for erythrocyte haemolysis and cell fusion. However, for the F glycoprotein to be optimally activated, the H glycoprotein is also required (Ruckle and Rogers, 1957; Choppin and Scheid, 1980; Vialard *et al.*, 1990; Wild *et al.*, 1991; Takenhara *et al.*, 1992; Cattaneo and Rose, 1993; Alkhatib *et al.*, 1994). For example, cell to cell fusion requires the

presence of both the H and F glycoproteins. A giant cell with many nuclei (multinucleated giant cell) occurs when infected cells fuse with uninfected cells. This can result in a syncytia comprising of 50 or more nuclei bounded by a single cytoplasmic membrane (Enders *et al.*, 1957). It has further been discovered that the treatment of cells with cytochalasin B prevents syncytia formation (Bedows *et al.*, 1983). Lysis of agglutinated red blood cells follows a few hours after haemagglutination (Peries and Chancy, 1962).

1.7.3 Cytopathic effects (CPE)

Cytopathic changes of three types accompany MV replication in cell culture monolayers of vero cells. These changes include the formation of multinucleated giant cells (syncytia), alteration of the cell shape and the formation of inclusion bodies (Enders *et al.*, 1957). The cells infected with MV may become stellate, dendritic or spindle in shape, and show increased refractility to light, compared to the normal polygonal shape they always display. This is normally the result of several stages of passaging (Milovanovic *et al.*, 1957; Seligman and Rapp, 1959). An observation that spindle cells occur particularly when high multiplicities of infection (Oddo et al., 1961) and complete medium (Reissig *et al.*, 1956) are used, has led to the speculation that several stages of passaging may be related to the production of defective interfering particles (Mckimm-Breschkin *et al.*, 1982).

1.7.4 Strain variation

MV virus is generally regarded as a stable virus. Sera from people who were infected decades ago easily neutralize the current wild-type MV strains. Those who have recovered from infection are immune for life (Griffin and Bellini, 1996). However, it is worth noting that the MV RNA-dependent RNA polymerase has an inherent error rate and no proofreading capacity, resulting in some degree of variability. Analysis of the nucleotide sequence of complementary DNAs has led to the description of such variabilities in the N, M, H and F genes (Taylor *et al.*, 1991; Rota *et al.*, 1992; Schulz *et al.*, 1992; Sakata *et al.*, 1993; Rota *et al.*, 1994; Rima *et al.*, 1995). Variability has also been described in the N, M and H proteins using monoclonal antibodies (Sheshberadaran and Norrby, 1986; Giraudon *et al.*, 1988; Tamin *et al.*, 1994).

1.7.5 Virulence

Wild-type MV strains have been found to be much more virulent than vaccine strains. For example, when marmosets or tamarins are exposed to these two types of MV strains, those infected with the wild-type strain die, while those infected with vaccine strains do not (Albrecht *et al.*, 1980; Lorenz and Albrecht, 1980). However, it is important to note that infection of humans and macaques do not usually result in fatalities. It is believed that attenuation of virulence is associated with decreased virus replication in lymphatic tissue (Kamahora and Nii, 1961; Ono *et al.*, 1970), diminished viraemia (Enders *et al.*, 1960; van Binnendijk *et al.*, 1994), and failure to induce pathologic changes in neural tissue (Buynak *et al.*, 1962).

1.8 Pathogenesis and Pathology

1.8.1 Classic Measles

1.8.1.1 Transmission

Anyone infected with MV can transmit infection from as early as three days prior to onset of symptoms, until the rash desquamates (Gordon and Ingalls, 1954). It has been discovered that infectivity is at its highest during the prodrome phase, and decreases rapidly with the onset of the rash. The virus is spread by a respiratory route. Direct contact with respiratory secretions and exposure to aerosols created by coughing and sneezing, predisposes one to infection (Riley *et al.*, 1978). In fact, aerosol spread is believed to be the most efficient way of transmitting measles. Also, a very low amount of infectious virus is sufficient to cause an infection in humans (Kress *et al.*, 1961).

1.8.1.2 Incubation Period

Measles has an incubation period of 9 to 11 days. However, some prefer to measure it from exposure to the onset of the rash, in which case, it becomes 10 to 14 days (Christensen *et al.*, 1952; Modlin, 1991). During this period, measles-related symptoms are not seen, except for the

blood counts which may reveal leukopenia due to a low number of circulating lymphocytes (Benjamin and Ward, 1932; Black and Sheridan, 1960). This is a clear demonstration of the fact that the incubation period, is a time of active virus replication and proliferation (Modlin, 1991).

1.8.1.3 Prodromal Phase

Following the incubation period, the patient enters a prodromal phase which may last from two to four days. This phase is characterized by fever plus signs and symptoms of upper respiratory tract infection, including cough, coryza and conjunctivitis. Body temperature may reach 39 - 40.5^0 C over a period of three to four days as fever becomes intense (Krugman *et al.*, 1977; Modlin, 1991; Carter and ter Meulen, 1995).

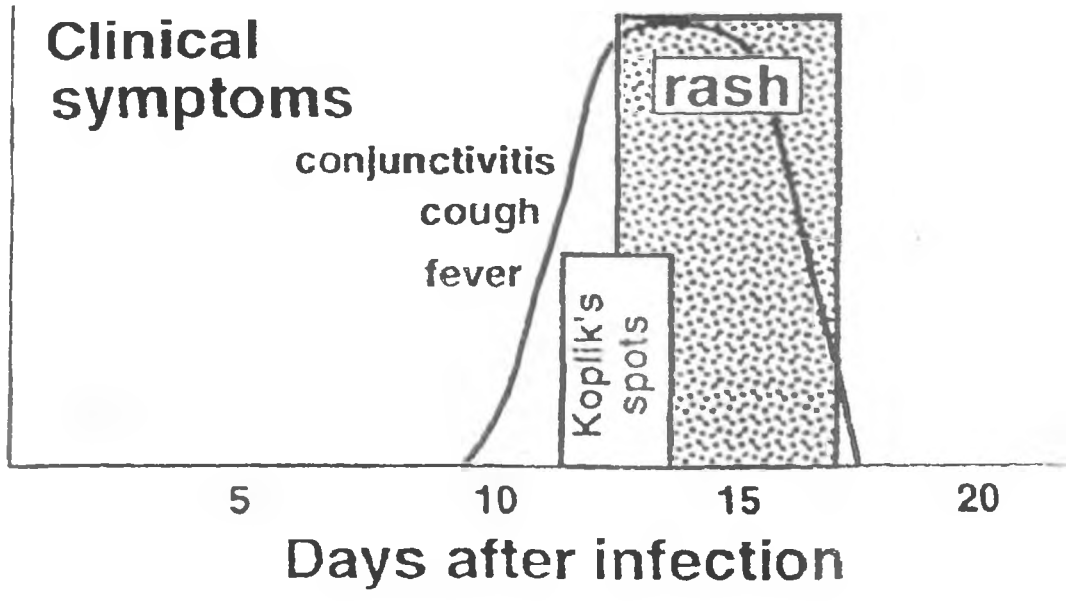


Fig. 1.2 : *Clinical symptoms of measles. Adapted from Griffin and Bellini, 1996*

The patient is highly infectious at this stage since the virus is present or can be detected in the blood and secretions (Modlin, 1991). Respiratory symptoms also increase in severity during this phase. Small irregular bright red spots with a minute bluish-white spot in the middle, appear on

the buccal mucosa and lower labial mucosa. They are called Koplik's spots and are a certain sign of measles (Koplik, 1896; Örvell, 1994; Carter and ter Meulen, 1995). These spots occur in more than 80% of cases (Gordon and Ingalls, 1954) and their presence is a useful diagnostic sign in the pre-eruptive phase of measles since they do not occur in any other illness (Modlin, 1991). These spots begin to fade two to four days after the onset of the prodromal phase as the rash develops (Modlin, 1991; Örvell, 1994; Carter and ter Meulen, 1995).

1.8.1.4 Rash

Fourteen days following exposure, a distinctive maculopapular rash appears, first on the forehead, then behind the ears, and on the upper part of the neck. It then spreads gradually down the body, reaching the lower limbs and feet within 2 to 3 days (Modlin, 1991; Örvell, 1994; Carter and ter Meulen, 1995). See Fig 1.2. The fever peaks on the second or third day of the rash, and then diminishes rapidly, signalling clinical improvement (Modlin, 1991). Once the entire body is covered, the rash begins to fade on the third day or fourth day, and a brownish discolouration occurs, sometimes accompanied by a fine desquamation (Carter and ter Meulen, 1995). The appearance of the rash is accompanied by a rapid decline in the amount of virus in the blood, such that MV is rarely recovered after the second day of the rash.

1.8.2 Atypical Measles

This type of measles is characterised by unusual clinical signs and symptoms, and is much more severe than classic measles. Signs and symptoms exhibited by people suffering from atypical measles include pneumonitis which is very severe and more frequent, severe skin lesions, and a high and more prolonged rash (Rauch and Schmidt, 1965; Nader *et al.*, 1968; Rod *et al.*, 1970; Young *et al.*, 1970; Brodsky, 1972; Gremillion and Crawford, 1981). It has been seen in the recipients of the inactivated measles vaccine used in the United States from 1963 to 1967 (Griffin and Bellini, 1996). Unlike in classic measles, the rash which is accompanied by evidence of haemorrhage or vesiculation, starts on the extremities and spreads to the trunk. Cases of atypical measles have been reported two to thirteen years after administration of formalin-inactivated vaccine (Welliver *et al.*, 1977; Hall and Hall, 1979; Martin *et al.*, 1979) and still may be occurring (Forni *et al.*, 1994).

1.8.3 Measles in the altered host

1.8.3.1 Immunosuppressed host

Measles can be prolonged or even be fatal in patients whose immunity is altered. These include patients who are or have been on chemotherapy or their immunity has declined due to malignancy or immunodeficiency. Severe measles has been reported in children with leukemia (Mitus *et al.*, 1959; Siegal *et al.*, 1977). Children infected with the human immunodeficiency virus (HIV) have been shown to be susceptible to severe and sometimes fatal measles infection, irrespective of whether they may have received live measles vaccine or recent immunoglobulin infusion (Linneman *et al.*, 1972; Centers for Disease Control, 1988).

Two studies reported a high death rate, due to measles, in infants infected with HIV (Diaz *et al.*, 1992; Oshitani *et al.*, 1996). It has also been noted that infection in early infancy is more common in these children (Embree *et al.*, 1992). The fact that measles can occur without the characteristic rash in HIV-infected individuals (Markowitz *et al.*, 1988), plus the fact that measles viral excretion can persist longer in these people (Scheifele and Forbes, 1972; Dossetor *et al.*, 1977), suggest that transmission could be facilitated, especially in health-care settings. Prevention of measles is also disturbed by the fact that measles vaccine is less immunogenic in HIV-infected children. In a study conducted by Arpadi *et al*, 1996, in the USA, it was reported that the serological response to vaccine appeared to be lower the older the child at vaccination.

Another study done to measure antibody response to a standard measles vaccine in children from former Zaire vaccinated at 9 months of age, revealed that antibody response in HIV-infected infants was significantly reduced than that found in non HIV-infected infants (Oxtoby *et al.*, 1989). Immunity in HIV-infected individuals appears to wane faster than in non-HIV-infected individuals (Al-Attar *et al.*, 1995), and this is worsen by the fact that HIV-infected people whose measles-specific antibody had waned, respond poorly to a repeat dose of measles vaccine (Arpadi *et al.*, 1996). Death in patients who are immunocompromised is caused by giant cell pneumonia which may occur at any time from the incubation period (Mitus *et al.*, 1959) up to a year following the rash (Siegal *et al.*, 1977). Live measles vaccine has also been responsible for fatal

giant cell pneumonia (Mitus *et al.*, 1962; Mawhinney *et al.*, 1971).

1.8.3.2 Measles in pregnancy

Once again, giant cell pneumonia has been described as the main cause of death during gestation. However, no link has been established between maternal measles and congenital anomalies (Dyer, 1940; Siegel, 1973; Jespersen *et al.*, 1977). Several studies done have, however, shown that acute measles induces labour in 22 to 37% of pregnant women, hence causing a high rate of spontaneous abortions and premature deliveries (Dyer, 1940; Siegel and Fuerst, 1966; Jespersen *et al.*, 1977; Gazela *et al.*, 1985). Kohn *et al*, (1933) and Gazela *et al*, (1985), report that measles virus can infect the foetus in utero. It has also been discovered a measles infected mother going into labour during the incubation period will, at the same time as the infant, develop a rash, suggesting transplacental infection late in gestation (Kohn, 1933; Dyer, 1940). Some of these infants may develop a severe disease with pneumonia (Dyer, 1940). Mortality is approximately 15%.

1.8.4 Subacute sclerosing panencephalitis (SSPE)

This is a very rare neurological disease, resulting from measles virus infection, affecting children and adolescents. Motor dysfunction, alteration in personality and mental deterioration, are the main characteristics of this disease. Other symptoms are convulsions, emaciation, coma, and death occurs within months to years following the onset of this disease (Freeman, 1969; Modlin, 1991). Males are much more affected by this disease than females. Modlin, *et al*, (1979), report that SSPE occurs 2 to 3 times more frequently in males than females, and 3 to 4 times more frequently in whites than blacks in the United States. Majority of children show symptoms between the age of 5 and 14 years. Children acquiring measles at an early age and those from rural areas have been shown to be at a high risk of developing SSPE. The reason as to why this is the case, is still unclear at this stage (Jabbour *et al.*, 1972; Modlin *et al.*, 1979). Rapid deterioration of intellectual function, which may manifest itself in different ways, such as personality change, forgetfullness, poor attention span, difficulty sleeping and emotional lability, are the first signs of this disease (Freeman, 1969; Jabbour *et al.*, 1969). In a few cases, there may

be some visual disturbances emanating from chorioretinitis (Robb and Walters, 1970; Font *et al.*, 1973). The patient may also exhibit sudden repetitive flexion movements in the extremities as often as 5 to 10 seconds. These movements, which do not interfere with the patient's state of consciousness, and which normally disappear with sleep, represent myoclonic seizures (Modlin, 1991). In the latter stages of the disease, cortical blindness occurs, and at this stage, intellectual function has been completely destroyed. The patient is at this stage showing signs of wasting, decerebrate posturing, as well as loss of bowel and bladder control. Dysfunctioning of the hypothalamus is indicated by abnormal vital signs, such as abnormal blood pressure and respiratory rate, irregular pulse and high temperature. At this stage death is inevitable (Modlin, 1991).

About 6 months to 3 years (mean : 24 months) following disease onset, death occurs (Case records of the Massachusetts General Hospital, 1974; Murphy *et al.*, 1974). Although this disease has been treated with numerous therapeutic procedures, antiviral drugs and biologic agents, none has altered the course of the disease (Modlin, 1991).

1.8.5 Complications

Secondary bacterial infections involving the upper and lower respiratory tracts, giant cell pneumonia, acute measles encephalitis and rarely SSPE, are some of the complications which can occur in the normal host (Modlin, 1991).

1.8.5.1 Respiratory complications

Acute measles may predispose the lungs to secondary bacterial infection by the following bacteria : *Streptococcus pneumoniae, Staphylococcus aureus* or *Neisseria meningitidis* (Lucke, 1918), and also by adenoviruses (Pather *et al.*, 1976). It is also important to note that MV is capable of directly infecting the pulmonary parenchyma, producing (Hecht's) giant cell pneumonia (Modlin, 1991).

1.8.5.2 Gastrointestinal complications

Gastrointestinal symptoms is a result of epithelial surfaces being infected with MV (Morley, 1969). Secondary bacterial and protozoal infections, are believed to be the cause of diarrhoea (De Mol *et al.*, 1984; Greenberg *et al.*, 1991), a common complication of measles (Arya *et al.*, 1987), especially in young children requiring hospitalization (Greenberg *et al.*, 1991).

1.8.5.3 Cardiovascular complications

Studies conducted by Cohen in 1963 and Finkle in 1964, indicate that death of the myocardium due to residual cardiac fibrosis of the conducting system (Giustra, 1954), occurs during the prodrome and acute phase of measles, and also some months later. Measles myopericarditis is also a complication of measles, although its occurrence is very rare. Degan, in 1937, discovered only two cases of measles myopericarditis and two additional cases of pericarditis in 100 autopsy specimens from patients who died during acute measles.

1.8.5.4 Neurological complications

Measles encephalitis complicates approximately one per 1000 cases of measles. This is an acute inflammatory reaction of the CNS associated with MV infection (Hoyne and Slotkowski, 1947; La Boccetta and Tornay, 1964; Measles encephalitis - United States, 1981). Vaccine administration is responsible for the decline in measles encephalitis which occurs in parallel to the declining incidence of measles, and as a result less than five cases are reported to the Centres for Disease Control each year (Measles encephalitis - United States, 1981).

1.9 Diagnosis

Most cases of measles are diagnosed on clinical grounds only. The following are clinical features characteristic of measles : fever (with temperatures of between 38.3^0 C and 40^0 C), erythematous maculopapular rash, coryza, cough, and Koplik's spots (Griffin and Bellini, 1996). Not all of the above signs and symptoms may be present in one particular case. The fact that many of these are

shared with other diseases, further serves to complicate matters (Makhene and Diaz, 1993). The CDC have devised their own clinical case definition of measles. It includes : a generalized maculopapular rash of 3 days or more; fever of 38,3^0 C (if measured); and one of the following signs and symptoms :- cough, coryza or conjunctivitis (Centers for Disease Control, 1983). The differential diagnosis includes scarlet fever, toxic shock syndrome, rubella, parvovirus B19 infection, meningococcemia, Kawasaki disease, dengue, and perhaps other as yet unidentified infections (Brown *et al.*, 1994).

MV can be isolated from a number of sources, such as throat swabs, conjunctival washings, sputum, urinary sediment cells and lymphocytes. Multinucleate giant cells with inclusion bodies are characteristic of measles. These cells are detectable in the nasopharyngeal secretions (NPS) during the prodromal phase (Carter and ter Meulen, 1995). The direct and indirect immunoflourescence (IF) have been used extensively. Both these techniques are applicable to cells shed in nasal secretions, although removal of antibodies already coating the virus antigens may be necessary. IF is useful for the diagnosis of measles in the pre-eruptive phase or in children vaccinated with killed vaccine, where rash development is atypical (Carter and ter Meulen, 1995).

Measles diagnosis is most often confirmed by serology. Enzyme immunoassay (EIA) is the best serological technique for the fact that it allows for differential detection of IgM and IgG, and its convenience when large numbers of samples are involved. As a result, this technique is widely used. Normally acute and convalescent serum pairs are the best samples to consider. Detection of MV-specific IgM antibodies in serum or saliva is diagnostic (Sabin, 1991; Perry *et al.*, 1993; Brown *et al.*, 1994) and may require a single sample (Griffin and Bellini, 1996). IgM antibodies appear at the time of the rash and can be detected 3 days after the onset of the rash in most individuals (Rossier *et al.*, 1991; Perry *et al.*, 1993).

1.10 Prevention and control

1.10.1 Prophylaxis

Immune serum globulin (ISG) is capable of preventing or modifying measles if given to children, up to six days following exposure (Ordman *et al.*, 1944). The target group should be children under 1 year of age, because of a high risk of complications in this age group. Intravenous immune globulin (IVIG), has also been used. This immune globulin contains a high titre of measles antibody. It has, however, been implicated in one case of fatal measles infection, after it had been regularly administered to an HIV-infected patient (Markowitz *et al.*, 1988).

1.10.2 Treatment

No standard antiviral treatment exists for measles. However, several studies report that Ribavirin has shown encouraging results, particularly in the reduction of the severity of symptoms in acute measles (Uylangco *et al.*, 1981), and inhibition of viral replication *in vitro* (Hosoya *et al.*, 1978; Murphy, 1978; Shigeta *et al.*, 1992). Some studies have also shown that when vitamin A is given in high doses during acute measles, even in the absence of clinical evidence of vitamin A deficiency, there is a significant decrease in morbidity and mortality (Fulginiti *et al.*, 1967; Barclay *et al.*, 1987; Coutsoudis *et al.*, 1991; Coutsoudis *et al.*, 1992).

1.10.3 Vaccination

1.10.3.1 History

Credit must go to Home (1759), for the his earliest attempts at vaccination against measles. From the observation that measles infection caused severe pulmonary symptoms, he concluded that the effects on the lung could be lessened by introducing the disease through the skin. There were other attempts between 1920 and 1940 by various groups of researchers, in which attempts were made at inactivating or culturing the virus in chick embryos. These, also, did not yield much success (Hektoen, 1905; Savini, 1923; O'Neil *et al.*, 1940; Ritossa and Mule, 1941). It is the

isolation of MV in culture which opened the way for a more concerted approach to vaccine development, with the Edmonston strain playing a key role (Enders and Peebles, 1954).

1.10.3.2 Vaccines

(A) Killed vaccine

This vaccine which contained the Edmonston MV strain, and commonly used in the United States of America, was obtained by subjecting the strain to formalin treatment with alum as an adjuvant (Warren and Gallian, 1962). Reports received from field trials, indicated that children who received this vaccine, lost antibodies to measles rapidly, and that the protection conferred by this vaccine waned over 3 years in 50% of the recipients (Fulginiti and Kempe, 1963; Winkelstein *et al.*, 1965). Some recipients acquired clinical infection, while some developed atypical measles syndrome upon exposure to natural measles (Karzon *et al.*, 1965 Rauch and Schimidt, 1965).

(B) Live-attenuated vaccine (Edmonston B)

Edmonston B vaccine, was the first live attenuated measles vaccine to be developed, by adaptation of the Edmonston MV strain to chick embryos, and subsequently to chick embryo fibroblasts. These were done after this strain was passaged 24 times in primary renal cells and primary amnion cells, (Katz *et al.*, 1958; Enders *et al.*, 1962). This vaccine was then tested in monkeys, where it induced antibody and protected the animals from subsequent challenges with low-passage Edmonston virus (Modlin, 1991). Seroconversion rates of more than 95% were recorded in trials conducted in institutionalized and home-dwelling children. Also antibody persistence and protection against natural infection were shown in these trials (Katz and Enders, 1959; Black and Sheridan, 1960; Katz *et al.*, 1960a; Katz *et al.*, 1960b; Kempe *et al.*, 1960; Kress *et al.*, 1961; McCrumb *et al.*, 1961; Katz *et al.*, 1962).

(C) Further live-attenuated vaccines

Schwarz vaccine, a more attenuated vaccine developed by Schwarz through further passaging

the Edmonston B virus in chick embryo fibroblasts (Schwartz, 1962), is currently the standard measles vaccine worldwide. This vaccine was licensed in 1965 (Griffin and Bellini, 1996). Another more attenuated vaccine, the Moraten strain which is closely related to the Schwarz, was licensed in US in 1968 (Rota *et al.*, 1994). Like the Schwarz strain, the Moraten strain was also obtained by additional passaging of the Edmonston B strain in chick embryo fibroblasts. Other further live-attenuated measles vaccine, also derived from the Edmonston B virus like Edmonston-Zagreb are used in certain parts of the world (Cutts *et al.*, 1994). This vaccine was obtained by passaging the Edmonston B strain 19 times in human diploid cells (Markowitz and Bernier, 1987).

1.11 Epidemiology

Measles occurs in every part of the world inhabited by man. This highly contagious communicable disease is transmitted efficiently through the respiratory route (Hope-Simpson, 1952). The absence of an animal reservoir or evidence of latent or epidemiologically significant persistent infection, implies that its maintenance in the population depends solely on the availability of susceptible people. It is primarily a childhood disease, the reason being that older individuals had been previously exposed to the virus, and as such have become immune to infection (Black, 1975). This disease can occur at any time of the year, although a marked seasonal variation has been noticed in endemic areas. In temperate climate, for example, it has been reported that morbidity due to measles increases during the months of October and November, the period of onset of cooler weather and the opening of schools. The opening of schools and indoor overcrowding during colder weather, provide opportunities for transmission (Gordon and Ingalls, 1954). Added to this is the fact that during winter, the lower humidity that prevails indoors, enhances the ability of measles to survive (De Jong and Winkler, 1964). It is reported to be very high between February and April (Modlin, 1991). Passively acquired maternal measles antibody protect the infant from measles during the first six months of its life. These antibodies begin to wane after this period, and the infant may later become susceptible to measles. Opportunity for contact with a contagious person determines the age of infection, beyond the first year of life. There is no correlation between susceptibility to measles virus and age (Modlin, 1991). Measles transmission depends on the number of individuals in a population and their opportunities for contact with the disease.

1.12 Immune responses

Several clinical manifestations of measles are due to immune responses to MV. These immune responses are important for two reasons, namely clearance of MV and recovery from infection. During the incubation period, the non-specific host immune responses play a major role in controlling the replication of the virus. MV-specific immune responses appear with the onset of clinical disease (Griffin and Bellini, 1996). At this stage, there is spontaneous proliferation of peripheral blood mononuclear cells (PBMCs) (Besser *et al.*, 1967; Ward *et al.*, 1990), polyclonal activation of B cells (Griffin *et al.*, 1985), expression of activation antigens on T cells (Griffin *et al.*, 1986), and increased plasma levels of cytokines and soluble surface proteins (Griffin *et al.*, 1989; Furukawa *et al.*, 1992; Griffin *et al.*, 1992). These are clear signs of activation of the immune system. At the same time as activation occurs, immune suppression appears, and both can continue for many weeks after recovery from measles (Griffin and Bellini, 1996).

1.12.1 MV-specific immune responses

1.12.1.1 Humoral immunity

In naturally infected people, MV antibodies are detectable in serum shortly after the onset of rash (Ruckle and Rogers, 1957). For those who have received a live attenuated measles vaccine, antibodies are detectable 11 to 14 days after vaccination (Krugman *et al.*, 1965). IgM antibodies are the first to be detected, and will last for 3 to 6 weeks (Schluederberg, 1965; Lievens and Brunell, 1986). IgG antibodies appear later than IgM antibodies, peak in 2 to 6 weeks, and gradually decline to low but persistent levels, for life. A boost in titre of IgG occurs during reinfection, but IgM antibodies do not reappear (Schluederberg, 1965). As a result, immune children exposed to natural infection would maintain higher levels of antibody as a result of subclinical reinfections, than do immune children who do not have opportunities for reinfection (Krugman *et al.*, 1965). Secretory IgA antibody is induced by both the natural infection and infection with attenuated vaccine virus (Bellanti *et al.*, 1969).

As far as induction of antibody production is concerned, the N protein seems to be the most effective as witnessed by the most abundant and most rapid production of antibody to this protein (Norrby and Gollmar, 1972; Graves *et al.*, 1984). Because of the abundance of this antibody, its absence is a reliable indicator of seronegativity (Griffin and Bellini, 1996). The M protein elicits only small amounts of antibody, except in atypical measles (Machamer *et al.*, 1980; Graves *et al.*, 1984). Antibodies to F protein may contribute to virus neutralisation by preventing fusion of the virus membrane with the cell membrane at the time of virus entry (Malvoisin and Wild, 1990). They can be measured in two ways; first by haemolysis of monkey erythrocytes by MV (haemolysin inhibition)(Norrby and Gollmar, 1972) or by immunoprecipitation of the protein (Norrby and Gollmar, 1975). Measurement of antibodies to the H protein is achieved by inhibition of monkey erythrocytes by MV (haemagglutination inhibition)(Norrby *et al.*, 1965; Giraudon and Wild, 1981). These antibodies are also capable of inhibiting haemolysis (Norrby and Gollmar, 1972; McFarlin *et al.*, 1980; Carter *et al.*, 1982) and are also the primary antibodies measured by neutralization of virus infectivity in tissue culture (McFarlin *et al.*, 1980; Giraudon and Wild, 1981; Giraudon and Wild, 1985), although antibodies to F do have some neutralizing activity as well (Varsanyi *et al.*, 1984).

In addition to protection from MV infection (Krugman, 1963; Albrecht *et al.*, 1977; Black, 1989; Siber *et al.*, 1993), antibodies contribute to recovery from infection (Lin and Hsu, 1986), and may also play a major role in establishing persistent infection (Rammohan *et al.*, 1982). Evidence suggests that when antibodies bind to infected cells, they alter the replication of virus in the cell and by so doing contribute to the control of infection (Fujinami and Oldstone, 1979; Fujinami and Oldstone, 1980; Schneider-Schaulies *et al.*, 1992). Therefore, failure to mount an adequate antibody response carries a poor prognosis (Wesley *et al.*, 1982).

1.12.1.2 Cell-mediated immunity

When lymphocytes from individuals immune to measles, are exposed to MV antigen (Graziano *et al.*, 1975; Gallagher *et al.*, 1981), they undergo transformation and kill MV-infected tissue culture cells (Labowskie *et al.*, 1974). According to Burnet (1968), the ability to recover from measles is an indication of the adequacy of T-lymphocyte-mediated immune responses. MV-

specific, HLA-restricted CD8+ cytotoxic T-lymphocytes (Kreth *et al.*, 1979; Sissons *et al.*, 1985; van Binnendijk *et al.*, 1990), and proliferating CD8+ T cells (Ward *et al.*, 1990) are detectable in blood at the time of the rash and in bronchoalveolar lavage fluid during pneumonitis (Myou *et al.*, 1993). When cells are infected with MV *in vitro*, MHC class I expression is up-regulated through the production of IFN-β (Dhib-Jalbut and Cowan, 1993), and if this process also takes place *in vivo*, it may enhance recognition of MV-infected cells by CD8+ T cells (Griffin and Bellini, 1996).

MV-infection activates CD4+ T cells as well. Proliferation of these CD4+ T cells occurs during the rash (Ward *et al.*, 1990), and soluble CD4 becomes elevated and remains so for several weeks (Griffin and Ward, 1993)(Fig.1.3). In people who are immune to measles, MV proteins can induce lymphocyte proliferation (Rose *et al.*, 1984; Pette *et al.*, 1993), and this proliferation has been shown to be MHC class II-restricted (CD4+ cells), and to occur in response to purified H N, P, F and M proteins (Bellini *et al.*, 1981; van Binnendijk *et al.*, 1993).

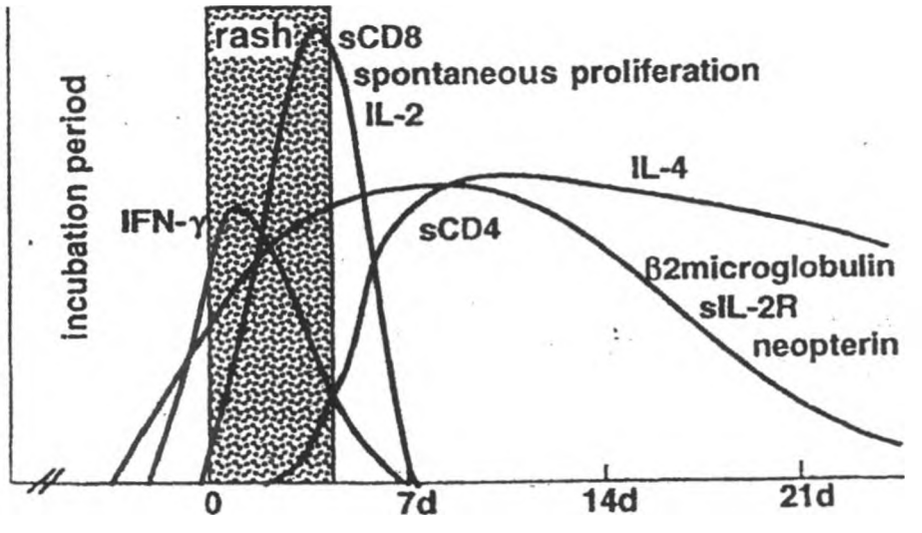


Fig.1.3 : *Levels of cytokines and soluble (s) cell surface molecules in the blood during uncomplicated measles. These patterns indicate early CD8+ and type 1 CD4+ T-cel activation, followed by type 2 CD4+ T-cell activation (see text). Adapted from Griffin and Bellini, 1996.*

As depicted in Fig. 1.3, circulating levels of cytokines produced by activated T cells and macrophages are high during measles. First to be elevated are IFN-γ, neopterin (a product of activated macrophages), and soluble IL-2 receptor. These are elevated during the prodrome, before the appearance of a rash (Griffin *et al.*, 1989; Griffin *et al.*, 1990). Next to be elevated, are IL-2, soluble CD4, and soluble CD8 at the time of the rash (Griffin *et al.*, 1989; Griffin *et al.*, 1992). IL-4 levels are raised as the rash fades, and they may remain elevated for weeks in some people (Griffin *et al.*, 1992).

1.12.1.3 Immunity to reinfection

Infection with MV confers lifelong immunity to symptomatic reinfection (Modlin, 1991). The long-term protection from reinfection following recovery from measles does not require re-exposure to the virus (Panum, 1939). Immunologic memory includes both continued production of antibody (Black and Rosen, 1962) and circulation of MV-specific T cells (Lucas *et al.*, 1982; van Binnendjik *et al.*, 1990; Wu *et al.*, 1993). Reports have been received of measles occurring in people previously infected with the virus, however, these are poorly documented (Watson, 1965). There have been reports of subclinical reinfection in children who have been infected naturally, or who are recipients of a measles vaccine (Krugman *et al.*, 1965; Linneman *et al.*, 1973). Two features clearly define subclinical reinfection. These are absence of IgM antibodies (Linneman *et al.*, 1973) and an anamnestic IgG antibody response (Linneman *et al.*, 1972).

1.12.1.4 Recovery from natural infection

The cell-mediated immune response appears to play a key role in recovery from MV infection. Failure to mount an adequate immune response, as seen in patients with Acquired immunodeficiency syndrome (AIDS) (Markowitz *et al.*, 1988; Centers for Disease Control, 1988), in congenital immunodeficiency syndromes (Nahmias *et al.*, 1967), immunosuppressive chemotherapy (Mitus *et al.*, 1959) and in the presence of malnutrition (O'Donovan, 1971) can lead to severe, and sometimes fatal, measles virus infections (Modlin, 1991). Good and Zak, (1956), and Ruckdeschel *et al* (1975), report that patients with isolated agammaglobulinaemia, and otherwise normal persons who have undetectable antibody responses to measles, handle

acute infection in the normal fashion and are immune to clinical reinfection.

1.12.1.5 Immune suppression

Measles is accompanied by immune suppression. This is supported by both *in vitro* and *in vivo* evidence. This suppression is thought to contribute to the susceptibility to secondary bacterial infections which have been implicated in most of the morbidity and mortality associated with MV infection (Miller, 1964; Morley, 1969; Beckford *et al.*, 1985). *In vitro* studies reveal abnormal lymphokine production (Joffe and Rabson, 1981; Crespi *et al.*, 1988; Ward *et al.*, 1991) and suppressed lymphoproliferative responses to mitogens (Arneborn and Biberfeld, 1983; Hirsch *et al.*, 1984). Further analysis of *in vitro* abnormalities has shown that there may be defects in the responses of both the monocytes and lymphocytes (Griffin and Bellini, 1996).

Several *in vivo* studies reported that delayed type hypersensitivity skin test responses to recall antigens, such as tuberculin, are suppressed before the rash and for several weeks after apparent recovery from infection (von Pirquet, 1908; Starr and Berkovich, 1964; Wesley *et al.*, 1978; Tamashiro *et al.*, 1987). There is also evidence of impairment of antibody production and cellular immune responses to new antigens (Coovadia *et al.*, 1974; Whitley *et al.*, 1982). Infection of monocytes by MV is believed to be responsible for the alteration of its function or cell lysis, or both (Esolen *et al.*, 1993; Griffin *et al.*, 1994). When macrophage lineage cells are infected with measles virus *in vitro*, IL-1 increases and tumour necrosis factor-α (TNF-α) decreases, implying that these abnormalities are a direct result of infection (Leopardi *et al.*, 1992). Deficient production of TNF-α, a proinflammatory cytokine, may contribute to decreased lymphoproliferation *in vitro*, and may blunt responses to new infectious challenges *in vivo* (Griffin *et al.*, 1992). T cell levels are low during measles, although normal proportions of CD4+ and CD8+ T cells are maintained (Arneborn and Biberfeld, 1983; Griffin *et al.*, 1985). One factor contributing to a low T cell count, is insufficient production of IL-2. This is supported by the observation that when recombinant IL-2 is added to cultures, there is spontaneous proliferation, and mitogen responses are improved (Griffin *et al.*, 1986; Ward and Griffin, 1993).

Changes have also been detected in the level of other cytokines such as IL-4 which may be raised compared to controls, and IFN-γ which may be low to normal (Crespi *et al.*, 1988; Ward *et al.*, 1991; Griffin and Ward, 1993). In addition to providing help for B cells, IL-4 stimulates growth and differentiation of type 2 CD4+ T cells but down-regulates the responses of type 1 CD4+ T cells and deactivates macrophages. In these ways, it serves as a general suppressant of macrophage activation and DTH responses and may be one of the mechanisms by which *in vivo* tuberculin responses and *in vitro* lymphoproliferative responses are suppressed during measles. These abnormalities of macrophage and T cell function may increase susceptibility to other infections (Griffin *et al.*, 1994).

1.12.1.6 Autoimmunity

About one in a thousand cases of measles may lead to encephalomyelitis. This usually occurs in older patients (Miller, 1964), about 14 days following the appearance of a rash (Litvak *et al.*, 1943). Johnson *et al.*(1984), report that the immune response to the myelin basic protein is induced, resulting in demyelination. This induction of the immune response to the myelin basic protein suggests that this is an autoimmune disease.

1.12.2 Non-specific immune responses

As mentioned earlier on, non-specific host immune responses play a major role in controlling viral replication during the incubation period. Furthermore, non-specific immune responses also play an important role in protecting the MV-infected host from secondary microbial infections.

1.12.2.1 Cytokines

Two cytokines, interferon-α (IFN-α) and interferon-β (IFN-β) are produced when cells are infected by MV *in vitro* (Volckaert-Vervliet and Billiau, 1977; Jacobson and McFarland, 1982). IFN-α slows down the replication of MV, and IFN-β increases the expression of the major histocompatibility complex (MHC) class I antigen on infected cells (Jacobson and McFarland, 1982; Dhib-Jalbut and Cowan, 1993). It has been reported that 8 to 11 days following

immunization, increased levels of IFN are present in serum (Petralli *et al.*, 1965), but during natural infection, elevated plasma levels of biologically active IFN have not been documented (Shiozawa *et al.* 1988; Griffin *et al.*, 1990).

1.12.2.2 Natural killer (NK) cells

Other cells worth mentioning, which may form part of the early defence mechanism, are the natural killer (NK) cells. Studies conducted on these cells indicate that their function is lower than normal during measles (Griffin *et al.*, 1990). However, these studies were done using human samples collected at the time of the rash and hence do not exclude induction of and roles for IFN and NK cells at earlier stages of infection (Griffin and Bellini, 1996).

1.12.2.3 Polymorphonuclear leukocytes (PMNL)

PMNL are the principal cells involved in the immune response to invasion of the host by bacteria and fungi (Abramson and Mills, 1988). Although the role of these cells in controlling viral infections is usually less important than that of mononuclear cells, the PMNLs are usually the first to be recruited to the site where there is invasion by microbes, and there they accumulate in large numbers, serving as the principal cell involved in the killing of these pathogenic organisms (Abramson and Wheeler, 1994).

These cells are also called polymorphonuclear granulocytes or granulocytes or polymorphs. As their name suggests, they have multilobed nuclei and contain many granules in their cytoplasm. They represent 60% to 70% of peripheral white blood cells. Their granules stain differently with histological dyes, and it is on this basis that they have been subdivided into neutrophils, eosinophils and basophils. Neutrophils are by far the most abundant, making up more than 90%-95% of circulating granulocytes. Hence the term polymorphonuclear leukocytes has become synonymous with neutrophils.

(A) Normal function of PMNL

As mentioned above, PMNLs are the first cells to arrive at the site of bacterial infection. Following invasion of the host by bacteria, chemotactic stimuli (e.g. bacterial peptides) are released and attach to specific receptors on the PMNL outer membrane. Occupation of receptors by a stimulus initiates the transduction of a signal, a sequence of biochemical steps collectively called the activation pathway. This in turn results in the end stage functions allowing for the ingestion, killing and degradation of the organism (Abramson and Wheeler, 1994). See Fig. 1.4 below.

There are a number of activation pathways and those that are activated depend on the specific type of receptor that is engaged by the stimulus. Engagement of a receptor by a ligand results in enhancement of G protein activity (G proteins link receptors to the initial enzyme that starts the activation pathway), changes in the concentrations of second messengers (e.g. cyclic adenosine 3',5'-monophosphate, calcium), phosphorylation/dephosphorylation of intracellular proteins and

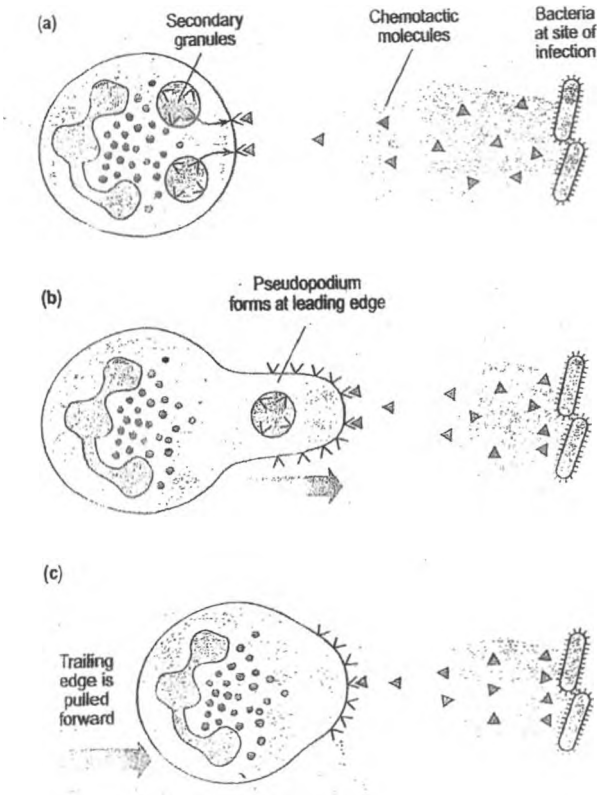


Fig. 1.4 : *Neutrophil chemotaxis. Adapted from Peakman and Vergani (1997).*

alterations in the structure of the cytoskeleton. Chemotactic activity occurs within one minute of the binding of the ligand to the receptor and is usually the first end stage function to occur. During the chemotactic process PMNL in the blood adhere to vascular endothelium and then migrate through the endothelial cell junction to the site of invasion.

Once at the infected area, opsonized microbes (Fig. 1.5) become bound to the cell by specific receptors (e.g. Fc antibody receptors and complement receptors). This new receptor-ligand interaction initiates an activation pathway similar although not identical to the signal induced by stimuli causing chemotaxis (Abramson and Wheeler, 1994).

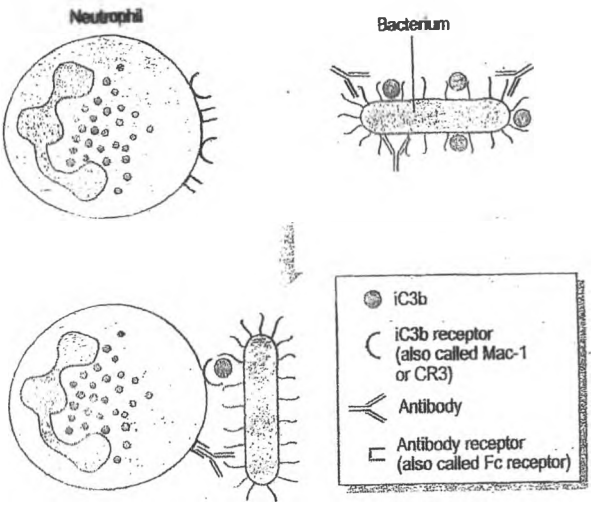
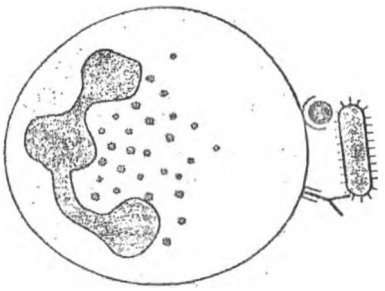


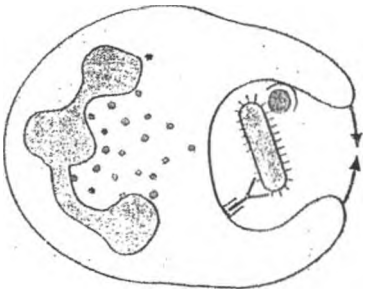
Fig. 1.5 : *Opsonisation. Opsonins coat microorganisms and enhance the neutrophils' ability to engulf them. Adapted from Peakman and Vergani (1997).*

Subsequently, the bacteria are phagocytosed, a process in which organisms are taken up in large intracellular plasma membrane-bound vacuoles known as phagosomes. Secretory activities then occur in which three types of lysosomal granules (i.e. primary, secondary and tertiary), each containing a variety of enzymes, fuse with phagosomes and other cellular plasma membrane sites. This process results in the release of enzymes into intracellular and extracellular spaces and causes killing and degradation of bacteria. Simultaneously, the oxidative burst is activated, producing oxygen radicals lethal to bacteria (Abramson and Wheeler, 1994). See Fig. 1.6.

(a)



(b)



(c)

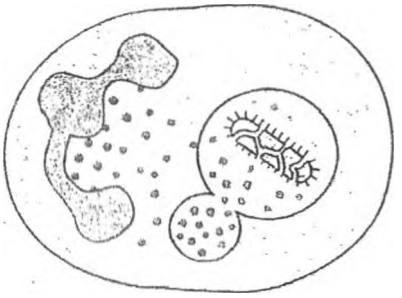


Fig. 1.6 : ***Phagocytosis : (a) Opsonized bacterium binds to neutrophil. (b) Pseudopodia extend around the bacterium. (c) Bacterium is engulfed inside a phagosome to which granules fuse and release their contents. Adapted from Peakman and Vergani (1997).***

(B) <u>Virus-induced alteration of PMNL function</u>

Evidence from previous studies, indicate that viruses can predispose humans to secondary and systemic bacterial and fungal diseases. Several viruses have been implicated in the depression of various functions of PMNLs. These viruses include Influenza, Cytomegalovirus (CMV), HIV, and many others (Abramson and Wheeler, 1994).

A large number of *in vitro* and *in vivo* studies involving animals and humans indicate that Influenza A virus has an immunosuppressive effect on PMNLs. PMNL dysfunction precedes and predisposes to the development of secondary disease in animals infected with Influenza A virus (Abramson et al., 1981; Abramson et al., 1982). In general, the studies have shown that Influenza A virus decreases PMNL oxidative, chemotactic, secretory and bactericidal activities whereas phagocytosis remains unaltered. These data support the concept that virus-induced neutrophil dysfunction has an important role in the development of superinfections (Abramson and Wheeler, 1994).

A report by Bale *et al.* (1982), indicated that PMNL functions are depressed in animals undergoing primary CMV infection. One study reported a decrease in the mobilization of PMNL to inflammatory sites in the guinea pig with primary CMV infection, which correlated with decreased release of hydrogen peroxide and decreased bacterial intracellular killing by peritoneal PMNL (Yourtee *et al.*, 1982). In addition, *de novo* appearance of both PMNL-directed and chemotaxin-directed plasma inhibitors has been noted during early CMV infection in guinea pigs (Tannous *et al.*, 1979).

HIV-induced PMNL dysfunction has been implicated in the increased risk of bacterial infections in HIV-1 infected individuals (Pitrak *et al.*, 1993). The variance in altered PMNL functions reported in several studies suggests that the type of PMNL dysfunction might depend on a number of factors including the stage of HIV disease and whether there are concomitant infections that also cause PMNL dysfunction (e.g. CMV) (Abramson and Wheeler, 1994).

(C) PMNL and Measles virus

Patients infected with MV, have been found to be at greatly increased risk of developing respiratory infections caused mainly by *Staphylococcus aureus* and *Mycobacterium tuberculosis* (McChesney and Oldstone, 1989; Aaby *et al.*, 1991). A team of researchers led by Anderson (Anderson *et al.*, 1976), reported that a more important cause of morbidity and mortality in patients with measles, was the secondary bacterial infection caused by bacteria against which PMNL are directed. These researchers demonstrated a severe defect of both directional and non-

directional movement of PMNL cells of all patients with measles. The chemotactic defect was not only demonstrated in an *in vitro* system, but was also substantiated by grossly impaired neutrophil accumulation in Rebuck skin windows. They further reported that this chemotactic defect in PMNL of patients with measles was not due to serum inhibitors. Without discounting the importance of local effects of the MV, it was suggested that a severe chemotactic defect, similar to the one they have described in their study, could play an important role in accounting for the frequency of occurrence and the severity of secondary bacterial infections (Anderson *et al.*, 1976).

1.13 Apoptosis

Apoptosis, also called programmed cell death, is the process whereby individual cells of multicellular organisms undergo systematic self-destruction in response to a wide variety of stimuli (Teodoro and Branton, 1997). Most, if not all animal cells have the ability to self-destruct by activation of an intrinsic cell suicide program when they are no longer needed or have become seriously damaged. The execution of this death program is often associated with characteristic morphological and biochemical changes (Wyllie *et al.*, 1980). Apoptosis is a genetically controlled preprogrammed event which eliminates cells during development when they have become redundant or which functions as an emergency response after radiation damage, viral infection, or aberrant growth induced by the activation of oncogenes. In the case of virus-infected cells, the induction of early cell death would severely limit virus production and reduce or eliminate spread of progeny virus in the host. Thus most animal viruses have evolved strategies to evade or delay early apoptosis to allow production of high yields of progeny virus (Teodoro and Branton, 1997).

A variety of physiological and non-physiological stimuli can provoke apoptotic cell death, among them, metabolic stimuli disturbances due to chemical insults, virus infections or developmental cues (Vaux and Strasser, 1996). Although not all signals which initiate the apoptosis pathway are understood, in many, but not all, cases the tumour suppressor protein p53 is required to propagate the signal to commit suicide (Levine, 1997). Members of the Bcl-2 family represent a major set point in the apoptotic pathway with cell fate resting, in many cases, with the dynamic

balance between family members which promote and inhibit apoptosis (White, 1996; Rao and White, 1997). Bcl-2-like family members appear to sit at a node in the apoptotic pathway at a point of integration for stimuli which provoke apoptosis and, in many but not all cases, appear to influence the activation of caspase family members [IL-1β-converting enzyme (ICE)-like proteases] which perform the 'execution' phase of apoptosis, cleaving a number of cellular proteins to bring about destruction of cell structure (Clem and Duckett, 1997; Nagata, 1997; Rao and White, 1997).

Apoptotic cell death is characterised by controlled autodigestion. Cells appear to initiate their own apoptotic death through the activation of endogenous proteases (Thompson, 1995). The process is characterized by a number of distinct morphological features and biochemical processes. These include cytoskeletal disruption, cell shrinkage and partial detachment from substratum and plasma membrane blebbing (Vaux and Strasser, 1996; White, 1996).

Apoptosis also involves characteristic changes within the nucleus. The nucleus undergoes condensation as endonucleases are activated and begin to degrade nuclear DNA. In many cell types, DNA is degraded into DNA fragments the size of oligonucleosomes, whereas in others larger DNA fragments are produced. Apoptosis is also characterised by a loss of mitochondrial function. This has led to speculation that mitochondria may have an important function in regulating apoptosis; however, data to support this hypothesis are currently unavailable.

The dying cell maintains its plasma membrane integrity. However, alterations in the plasma membrane of apoptotic cells signal neighbouring phagocytic cells to engulf them and thus complete the degradation process (Thompson, 1995). Cells not immediately phagocytosed break down into smaller membrane-bound fragments called apoptotic bodies. An important feature of apoptosis is that it results in the elimination of the dying cell without induction of an inflammatory response (Thompson, 1995).

1.14 Objectives of this study

MV-infected persons have been found to be at a greatly increased risk of developing respiratory infections caused mainly by *Staphylococcus aureus* and *Mycobacterium tuberculosis.* PMNL play an important role in combating primary and secondary bacterial and fungal infections. It has been reported that secondary bacterial infections are an important cause of morbidity and mortality in patients with measles.

The only study we know of, to date, that has addressed the effects of MV on PMNL, is the one conducted here in South Africa by a group of researchers led by Anderson (Anderson *et al.*, 1976). Neutrophils isolated from MV-infected children were shown to be defective for chemotaxis. It was, however, not established in that study whether MV, directly or indirectly, was the cause of this defect.

The main question being addressed in this study is whether MV has any direct effects on PMNL functions that could account for the increased susceptibility of MV-infected persons to secondary microbial infections.

The objectives of this study, therefore, were :-

(a) To determine which, if any, PMNL functions (i.e. chemotaxis, phagocytosis, degranulation and oxidative burst), are altered by exposure to MV *in vitro.*

(b) To determine whether MV can, directly or indirectly, induce apoptosis *in vitro.*

(c) To determine whether virus attenuation or heat inactivation of MV strains have any effect on these functions.

(d) To determine whether ligation of the MV-specific receptor, CD46, by CD46 antibody can alter any of the PMNL functions mentioned above.

CHAPTER 2

MATERIALS AND METHODS

2.1 PREPARATION OF VIRAL STOCKS

Reagents

Viral seed stocks
Cell line (Vero)
RPMI 1640
Foetal Calf Serum (FCS)

Viral seed stocks and vero cells that were used for the purpose of this study were obtained from the Measles Unit of the National Institute for Virology (NIV). The stocks had been stored in 1 ml aliquots at -70^{0}C.

2.1.1 Trypsinization of vero cells

Vero cells, once confluent were trypsinized as follows : the supernatant was poured off a T150 flask (tissue culture flask with area of 150 cm^2) containing vero cells. 5ml of trypsin was added to the flask to loosen cells attached to the flask. The flask was then placed in the hood for 5 minutes, after which 8 ml of medium (RPMI 1640 + 4% FCS) was added to wash off the cells and trypsin. The contents of the flask were then transferred to a falcon tube, and centrifuged in a Sigma 4K15 centrifuge (Laborzentrifugen GmbH 37520 Osterode)for 5 minutes at 1000 rpm (rotor 11150). After discarding the supernatant, the pellet was resuspended in 14 ml RPMI 1640 + 4% FCS. To each of the 4 T75 flasks, 4 ml of the cell suspension was added, and made up to 35 ml, in each flask, with RPMI 1640 + 4% FCS. The flasks were then placed in an incubator at 37^0 C (5% CO_2), and monitored over a number of days for confluency.

2.1.2 Inoculation of vero cells with MV strains

Three different measles virus strains representing different degrees of attenuation were used for cell inoculations. These were the Schwartz (SCH) strain (a vaccine strain), Johannesburg 25 (JHB 25) strain (a vaccine-like strain) and a strain designated D1317 (a wild-type strain). After establishing that the vero cells were almost confluent, the supernatant was poured off, and 1 ml of the virus stock added. The supernatant in another flask (uninfected control) was discarded and fresh medium added. The cells were then placed in an incubator at 37^0 C (5% CO_2), and checked daily for cytopathic effect (CPE) over a number of days. MV infections were graded according to the CPE of the cells at the time of harvesting. The grading was as follows : + = CPE of about 20% - 30%; ++ = CPE of 50% - 60%; +++ = CPE of 70% - 80% , and ++++ = CPE of 90% - 100%.

2.1.3 Harvesting of viral stocks

Virus stocks were immediately harvested when all flasks of cells for each MV strain showed a CPE of ++++ (90% - 100%). This was done to ensure that all stocks of virus strains were approximately similar in concentration. That stock viruses were of equivalent infectivity, was confirmed by the fact that when used at the same dilution to infect vero cells, gave rise to the production of CPE at a similar rate. Cells still attached to the flask were gently scraped off with a cell scraper. The contents of each flask were transferred to a 50 ml falcon tube, and centrifuged at 1000 rpm, for 5 minutes, at 4^0 C. The supernatants were then discarded, and the pellet in each tube was resuspended in 14 ml RPMI 1640 + 4% FCS. These cell suspensions were then stored in aliquots of 1 ml in 1.5 ml nunc tubes, at -70^0 C.

2.1.4 Heat-inactivation of viral stock preparations

A proportion of each of the viral stocks prepared above were incubated in a 56^0 C water bath for 1 hour. They were then removed and stored at -70^0 C in 100 ul aliquots. The heat-inactivated preparations of Schwartz, Johannesburg 25 and D1317 strains, were designated Schwatz-HI (Sch-HI), Johannesburg 25-HI (JHB 25-HI) and D1317-HI respectively (where the suffix HI

indicates heat inactivated).

2.2 ISOLATION OF PMNLs FROM WHOLE BLOOD

Reagents

Blood units (from SABTS, Johannesburg)

Phosphate Buffered Saline (PBS), pH 7.2 (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 20mM NaH_2PO_4)

Ficoll Hystopaque®-1077 (Sigma Diagnostics Inc., St. Louis, USA)

FCS

Ammonium chloride, pH 7.2 (0.15M NH_4Cl, 10mM $KHCO_3$, 1mM sodium EDTA)

Trypan blue [0.5% (w/v)]

Units (500ml) of whole anticoagulated (acid citrate dextrose) blood were obtained from the South African Blood Transfusion Services (SABTS), Johannesburg. A portion of the blood volume was processed and all experiments conducted within 24 hours of blood being taken.

PMNLs were isolated from whole blood using Ficoll-Histopaque®-1077. Two 50 ml falcon tubes were used, and 20 ml of blood was added to each tube. An equal volume of PBS was added, and mixed with blood in both tubes. A further four 50 ml falcon tubes were used, and 15 ml of Ficoll Histopaque was added to each one of them. Then, 20 ml of PBS-diluted blood was layered onto ficoll hystopaque in each tube. The tubes were centrifuged at 2000 rpm for 30 minutes at room temperature. Four layers could be detected after this in the following order from top to bottom : plasma, peripheral blood mononuclear cells (PBMCs), ficoll-histopaque and finally PMNLs just above the red blood cells (RBCs). The top three layers were removed and discarded, and the PMNL layer (with a small volume of RBCs) was placed in a clean falcon tube. An equal volume of PBS was added. Ficoll hystopaque was added to two falcon tubes (15 ml in each tube), and 20 ml of the mixture was layered onto this ficoll hystopaque. The tubes were centrifuged at 2000 rpm for 30 minutes at room temperature. At the end of this, the PMNL layer appeared as a cell button above the RBCs. Other layers were discarded and the PMNL layer was transferred to a

clean tube. Contaminating RBCs were lysed with ammonium chloride until all RBCs were lysed. The pellet was washed twice with PBS, following this lysis, and then resuspended in RPMI 1640 + 10% FCS. A cell count was determined by trypan blue exclusion dye, a stain which discerns live from dead cells. Percentage viability as well as total cell counts were calculated from this, and only PMNL preparations with a viability of 95% or more were used in this study.

2.2.1 Inoculation of PMNLs with MV strains

Reagents

VIRAL STOCKS :
vaccine : Schwartz (Sch) and
heat-inactivated Sch (Sch-HI),
vaccine-like : Johannesburg 25 (Jhb 25) and
heat-inactivated Jhb 25
wild-type : D1317 and
heat-inactivated D1317
PBS
RPMI 1640
FCS

The concentration of PMNLs was first adjusted to 1 x 10^7 cells/ml in RPMI. To each of the 7 clearly marked eppendorf tubes, 1 ml of cell suspension was added. Viral stocks were added to the respective tubes and PBS to the control tube (final concentration of 10%), and then mixed with the cell suspension by gentle vortexing. The strains mentioned above (under reagents) were used to inoculate the PMNLs. PBS was found to give the same results as medium from control grown vero cells, and was therefore used throughout this study. Following inoculations, the tubes containing inoculated PMNLs were incubated at 37^0 C (5% CO_2) for 1 hour, after which they were briefly centrifuged in a microcentrifuge (centrifuge 5415 C), to pellet the cells. The supernatant containing unattached virus was gently removed by pipetting, cells washed with PBS and the resultant pellet resuspended in 1 ml RPMI 1640 + 10% FCS.

2.3 ASSAYS OF PMNL FUNCTIONS

2.3.1 Chemotaxis

Reagents

Interleukin-8 (IL-8)
RPMI 1640 + 10% FCS
anti-CD46 antibody
Trypan blue

The aim of this test was to measure and compare the migration of PMNLs that have been exposed to MV, both live and heat-inactivated, in response to a chemoattractant, IL-8. PMNLs were infected with strains as described 2.2.1. Only RPMI + 10% FCS was added to the control wells, while the test wells had IL-8 added to them in addition to RPMI + 10% FCS. Transwell inserts (Corning costar, 6.5 mm in diameter, and a pore size of 3 um), were placed in the wells of a 24-well plate and allowed to equilibrate for 5 minutes. 100 ul of each cell sample was added to each of two wells. The plate was then incubated for 1 hour at 37^0 C (5% CO_2). The inserts were removed and the cells in each bottom well thoroughly resuspended with a pipette and counted using trypan blue exclusion.

2.3.2 Phagocytosis

Reagents

Phagotest kit® (Orpegen Pharma, Heidelberg, Germany)
Human serum (Bio Whittaker, Maryland, USA)
RPMI 1640

The ability of PMNLs, in the samples mentioned in the preceding sections, to engulf microorganisms was measured using a Phagotest kit® (Orpegen Pharma, Heidelberg, Germany),

with a slight modification to the experimental procedure as detailed in the instruction manual. The test kit contains fluorescein (FITC)-labeled opsonized bacteria (*E. coli*-FITC) and necessary reagents to measure phagocytosis and allows for the quantitative determination of leukocyte phagocytosis in heparinized whole blood. The experimental procedure was therefore modified to allow the quantitative determination of leukocyte phagocytosis in PMNLs isolated from whole blood. This assay measures the overall percentage of PMNLs showing phagocytosis (ingestion of one or more bacteria per cell) and the capacity of individual cells to phagocytose *E. coli* (number of bacteria per cell or fluorescence intensity).

PMNLs were isolated and infected as described in earlier sections. The PMNLs in all samples were then resuspended in RPMI + 50% human serum (instead of FCS). The reason for this step was to maximise the opsonization of bacteria by PMNLs. Two tubes were used for each sample (i.e. the control and test tube). To the designated tubes, 100 ul of the cell suspension from the relevant samples, was added. The tubes were incubated in an ice bath for 10 minutes, to cool them down to 0^0 C. Following the cooling, the *E. coli* bacteria, which had also been cooled in the waterbath, were vortexed and 20 ul added to each tube. The samples were all mixed and the test samples incubated at 37^0 C in a waterbath for 20 minutes, while the control samples remained on ice (to prevent phagocytosis). Both the incubation times and temperature were closely monitored as these are critical to the success of the assay.

Precisely at the end of the incubation period all samples together on one rack were simultaneously taken out of the water bath and placed on ice in order to stop phagocytosis. To each of the tubes, 100 ul of quenching solution was added, and the samples vortexed. The contents of each tube were then washed three times for 5 minutes at 1000 rpm at room temperature with a washing solution. Two hundred microlitre (200 ul) of a DNA staining solution was added to each tube, vortexed, and incubated in an ice bath protected from light for 20 minutes. A FACSort Flow Cytometer with a 488 nm argon laser (Becton Dickinson, California, USA) and Cellquest™ (version 1.0) software, were used to analyse fluorescence, within 60 minutes of completion of the assay. The results are expressed as the percentage of PMNL within the granulocyte gate that engulf *E .coli* and as fluorescence intensity or median channel shift (determined as median channel number (MCN) obtained for each test sample minus

the MCN obtained with the negative control tube).

2.3.3 Oxidative Burst

Reagents

Bursttest™kit (Orpegen Pharma, Heidelberg, Germany)
Human serum (Bio Whittaker, Maryland, USA)
RPMI 1640

The oxidative burst of PMNLs in various samples, was measured in response to *E. coli*, using the Bursttest™ kit (Orpegen Pharma, Heidelberg, Germany). This assay allows for the quantitative determination of leukocyte oxidative burst in heparinized whole blood. The experimental procedure was modified to allow for the quantitative determination of oxidative burst in PMNL isolated from whole blood. The kit contains unlabeled opsonized bacteria (*E. coli*), dihydrorhodamine (DHR) 123 as a fluorogenic substrate and necessary reagents. The assay determines the percentage of phagocytic cells which produce reactive oxidants (conversion of DHR 123 to rhodamine (R 123)) and their enzymatic activity (amount of R 123 per cell or fluorescence intensity).

PMNLs were isolated and infected as described in the preceeding sections. As with phagocytosis, PMNLs in all samples were resuspended in RPMI + 50% human serum (instead of FCS), to maximize opsonization of bacteria. Two tubes (control and test) for each sample were used. To each tube, 100 ul of the relevant sample was added, and the tubes were incubated in an ice bath for 10 minutes, to cool them down to 0^0 C. Precooled *E. coli* bacteria were then mixed well and 20 ul added to test tubes only. To the control tubes, 20 ul of washing solution was added. All samples were mixed, and then incubated in a closed preheated water bath at 37^0 C for 20 minutes. Both the incubation times and temperature were closely monitored. After 20 minutes, all the tubes in one rack were put back in an ice bath, and 20 ul of substrate solution was added to each tube. The tubes were again incubated for 20 minutes in a water bath, and then washed once with 3 ml washing solution for 5 minutes at 1000 rpm. The supernatant was discarded and 200 ul

DNA staining solution was added to each tube. After vortexing, the tubes were incubated in an ice bath, protected from light, for 20 minutes, after which the evaluation of oxidative burst was done by flow cytometry. The results are expressed as the percentage of PMNL within the granulocyte gate that produce reactive oxygen intermediates and as fluorescence intensity or median channel shift (determined as median channel number (MCN) obtained for each test sample minus the MCN obtained with the negative control tube).

2.3.4 Degranulation

Reagents

0.01 M p-nitrophenyl-β-D-glucuronide
0.1 M sodium acetate (pH 4)
0.4 M glycine buffer (pH 10)
Cytochalasin B
Triton-X100
IL-8
PBS

The aim of this test was to measure and compare the release of a hydrolase, β-glucuronidase from azurophilic granules of PMNLs exposed to MV. PMNLs in these samples had been earlier incubated for 1 hour at 37^0 C (5% CO_2), with the three MV strains, and their heat-inactivated preparations, with PBS used as a control. A volume of cells sufficient for the test from each sample, was first incubated with Cytochalasin B for 15 minutes at a temperature of 37^0 C before being placed in the respective wells of a round bottom 96-well plate. Four wells were used for each sample, and 100 ul of cell suspension was added to each well.

For each sample, the first well was the control well for spontaneous release (PBS), the second the test well (IL-8 concentration 500 ng/ml) well, the third as the test well (IL-8 concentration 62.5 ng/ml) and the fourth one as the control well control for total enzyme release (50% Triton X). After the above reagents had been added to these wells, the plate was incubated at 37^0 C (5%

CO_2) for 30 minutes. The plate was then centrifuged at 1000 rpm for 10 minutes at 4^0 C. The supernatant was collected and transferred to a flat bottom 96-well plate. To this, 100 ul of 0.01 M p-nitrophenyl-β-D-glucoronide in 0.1 M sodium acetate (pH 4) was added. The plate was left overnight at 20^0 C after which the reaction was stopped with 100 ul of 0.4 M glycine buffer (pH 10) in each well, and the absorbance was read at 405 nm. The results are expressed as the percentage of enzyme released calculated as : [OD_{405nm} in control or test wells/ OD_{405nm} in Triton-X100 lysed PMNL (total enzyme)] X 100.

2.4 APOPTOSIS ASSAY

A Cell Death Detection ELISA^{PLUS} (Boehringer Mannheim, GmbH, Germany) was used to quantify histone-associated DNA fragments (mono- and oligonucleosomes) present in the cytoplasm of cells undergoing apoptosis.

PMNLs were isolated and infected as described in the preceeding sections, and suspended in RPMI + 10% FCS. A round bottom 96-well plate was used, and 200 ul of the cell suspension from each of the samples (PBS, Sch, Sch-HI, Jhb 25, Jhb 25-HI, D1317, and D1317-HI), was added to the plate. After the plate had been centrifuged at 1000 rpm at room temperature, for 10 minutes, the medium supernatant was aspirated from each well and discarded. To the remaining cell pellet, 200 ul of lysis buffer (provided in the kit) was added, and the plate was left for 30 minutes at room temperature. The plate was then centrifuged at 1000 rpm for 10 minutes at room temperature. From each well, 20 ul of the lysate was transferred into the wells of the ELISA-microtitre plate. The lysates were incubated with 80 ul of an immunoreagent. The plate was incubated for 2 hours at room temperature, after which it was washed 3 times with 250 ul of incubation buffer in each well. A substrate solution (100 ul), also prepared according to instruction in the manual, was added to each well, and the plate was incubated at room temperature for 15 minutes. The absorbance was then read at 405 nm, using a plate reader (Organ Teknika Reader 230 S (Anthos Labotech Instruments GmbH , Austria).

2.5 LIGATION OF THE MV RECEPTOR BY ANTI-CD46 ANTIBODY

The aim of this test was to determine whether ligation of the CD46 receptor by an antibody that specifically binds this receptor has any effects on PMNL functions already mentioned in the preceeding sections. Two PMNL functions, chemotaxis and apoptosis, were chosen for the purpose of this investigation. The ability of CD46 antibody to affect IL-8 induced chemotaxis and to induce apoptosis were therefore studied.

Instead of inoculating PMNL with MV strains or their heat-inactivated forms, the MV-specific receptors, (CD46 receptors), on these cells were ligated by the addition of CD46-specific antibody. Two different concentrations of the CD46 antibody and of the isotype control IgG_1 antibody were used. The assays for these PMNL functions were then performed as described in sections 2.3.1 and 2.4.

2.6 STATISTICAL ANALYSIS

The SPSS statistical package (SPSS version 10.0 for Windows) was used for the determination of statistical significance of results. Comparison of means obtained in various PMNL functional assays using uninfected PMNL, PMNL exposed MV strains, their heat-inactivated forms, as well as the two antibodies mentioned, was done using a two-tailed T-test. The results were considered statistically significant when $p < 0.05$. All bar graphs show mean values (height of solid bars) from multiple determinants and standard errors of the mean (SEM). Line graphs (degranulation sections 3.1.4.1 (b) and 3.1.4.2 (b)) show mean values of multiple determinations at two input concentrations of IL-8.

CHAPTER 3

RESULTS

3.1 MV AND PMNL FUNCTIONS

3.1.1 Chemotaxis

3.1.1.1 Exposure of PMNL to MV strains and their ability to migrate in response to IL-8

The ability of PMNL exposed to the different MV strains to migrate in the absence (random migration) and in the presence (direct migration) of a chemoattractant was assessed, using PMNL isolated from the blood of 5 healthy individuals.

Random migration of PMNL was not significantly affected by exposure to any of the MV strains relative to the uninfected control ($p > 0.05$). The response of PMNL to IL-8 was measured as the number of cells that migrated across the membrane and were expressed as chemotactic indices (that is, the total number of cells that migrated in response to IL-8 divided by the number of cells that migrated randomly). The results are depicted in Figure 3.1 on the next page.

The chemotactic index of PMNL exposed to each of the three MV strains, was significantly reduced relative to that of the uninfected control ($p < 0.05$). The reduction in IL-8 induced chemotaxis, relative to the uninfected control, occurred to the greatest extent (53%) when PMNL were exposed to the wild-type D1317 strain. The decreases observed due to the Schwartz (vaccine) and Jhb 25 (vaccine-like) strains were by 45% and 41% relative to the uninfected control, respectively (see Fig. 3.1).

The chemotactic index of the Jhb 25-exposed PMNL was 20% higher than that of the D1317-exposed PMNL, the only difference, between strains that was significant ($p < 0.05$).

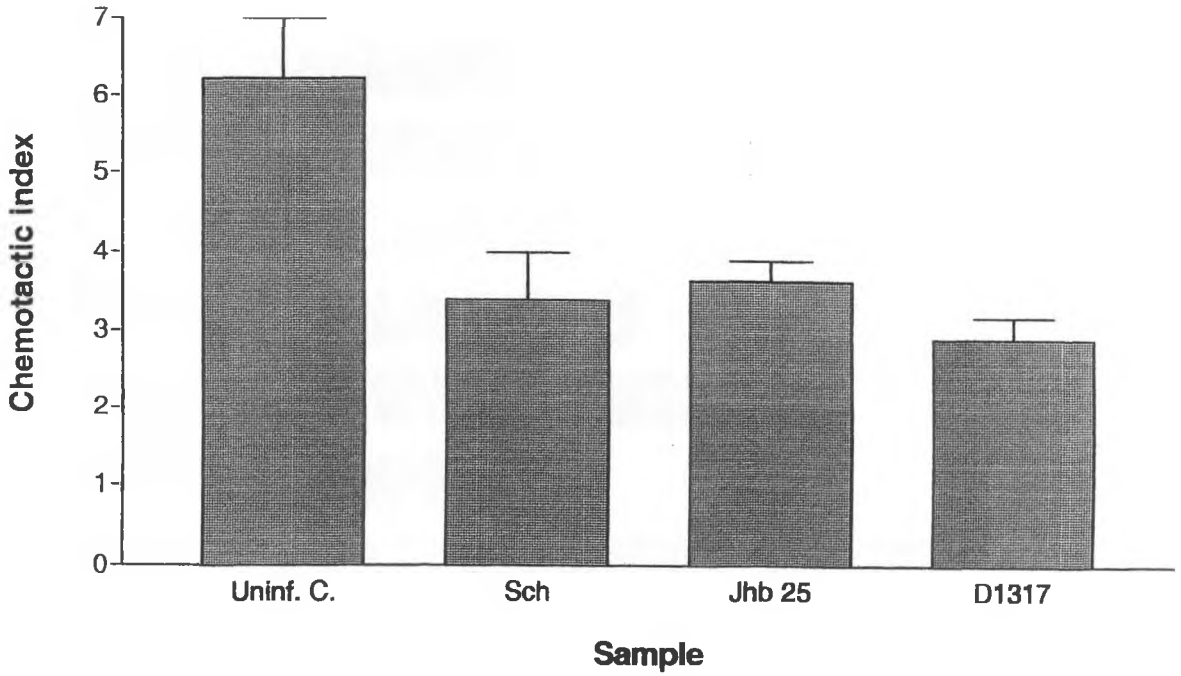


Figure 3.1 : *Chemotactic index : uninfected PMNL compared to MV-exposed PMNL. Uninf. C. = uninfected control; Sch = Schwartz (vaccine strain); Jhb 25 = Johannesburg 25 (vaccine-like strain) and D1317 = wild-type strain.*

3.1.1.2 The effect of heat-inactivation of MV on the ability of MV-exposed PMNL to migrate in response to IL-8

All three MV strains were inactivated by exposure to a temperature of 56^0 C for one hour for the purpose of determining how heat inactivation of the MV strains would affect PMNL migration in response to IL-8. The results are shown in Figure 3.2.

Heat inactivation of each of the MV strains, although accompanied by small increases in PMNL chemotaxis relative to that of the untreated MV strains, failed to bring about a significant recovery of function to that of uninfected levels. A comparison of chemotactic indices of the three MV strains and their heat-inactivated forms showed no significant increases in the chemotactic indices ($p > 0.05$), and all indices were significantly decreased when compared to the uninfected control ($p < 0.05$).

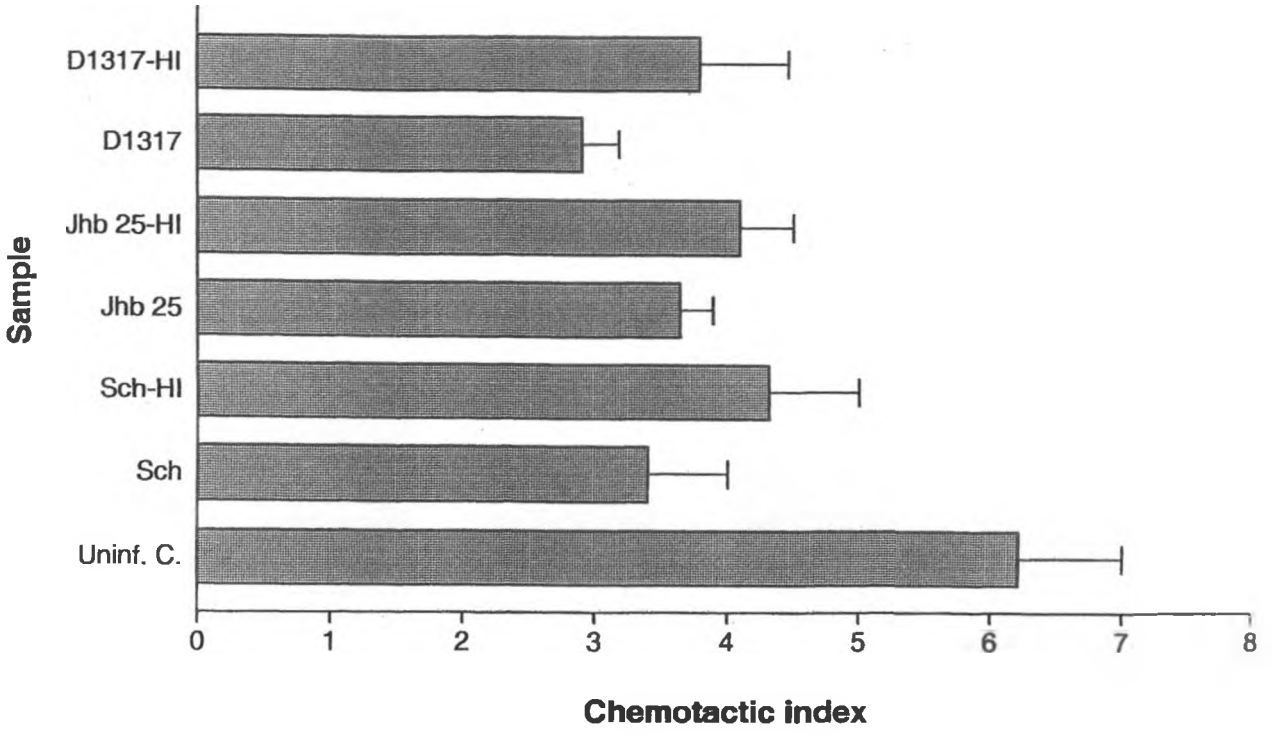


Figure 3.2 : *Chemotactic indices of PMNL exposed to both the untreated and the heat-inactivated forms (HI) of the three MV strains used are compared with that of the uninfected control, i.e. PMNL exposed to PBS.*

3.1.2 Phagocytosis

The ability of PMNL exposed to the different MV strains, viz. :- the Schwartz (vaccine), the Jhb 25 (vaccine-like) and the D1317 (wild-type) MV strains, to phagocytose bacteria was assessed using PMNL isolated from the blood of 7 healthy individuals. Both the percentage of PMNL having ingested fluorescent bacteria and the number of ingested bacteria per individual cell (flourescence intensity or median channel shift), were determined by flow cytometry. The results are shown in the bar graphs of Figures 3.3 and 3.4.

3.1.2.1 PMNL exposure to MV strains and their ability to phagocytose bacteria

In all samples in which PMNL were exposed to the three MV strains mentioned above, the

percentage of PMNL capable of undergoing phagocytosis was found to be less than that of the uninfected control (Fig. 3.3A). However, this reduction was only significant in the Schwartz- and D1317-exposed PMNL ($p < 0.05$), where the percentages of PMNL having performed

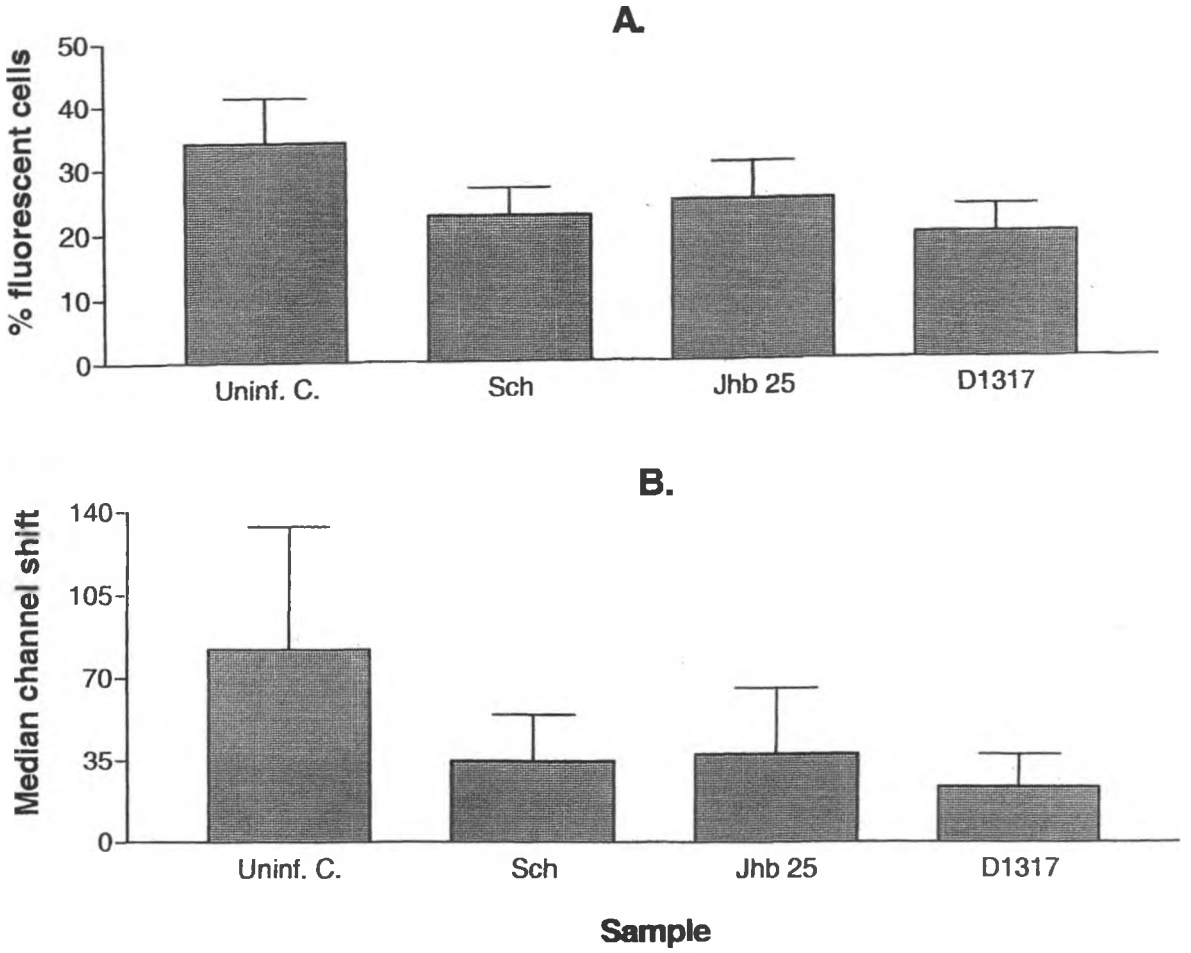


Figure 3.3 : ***A.*** *Percentage fluorescent cells (percentage of PMNL having phagocytosed bacteria).* ***B.*** *Fluorescence intensity(median channel shift).*

phagocytosis were 22.6% and 19.5% respectively, compared to 34.1% in the uninfected control. Effectively, these were reduced by 34% (Schwartz) and 43% (D1317) relative to the uninfected control. In the Jhb 25-exposed PMNL, 25% of cells were capable of phagocytosis, but the reduction in phagocytosis was not significant when compared to the uninfected control ($p > 0.05$)

MV-exposed PMNL also showed a reduction in the number of ingested bacteria per individual cell (measured as median channel shifts) when compared to the uninfected control (Fig. 3.3B). A decrease of more than 50% in the median channel shift in each of the three MV-exposed PMNL was observed, with the D1317-exposed PMNL showing the greatest reduction (71%). The reductions with the other two strains were 58% (Schwartz) and 54% (Jhb 25). See Fig. 3.3B.

Comparison of the median channel shifts of the MV-exposed PMNL, revealed the same trends as in for percentage of fluorescent cells. The highest median channel shift was observed in the Jhb 25-exposed PMNL. The medium channel shift measured in the Schwartz-exposed PMNL was slightly lower than that of the Jhb 25-exposed PMNL ($p > 0.05$), and 47% higher than that of the D1317-exposed PMNL ($p < 0.05$). Comparison of the median channel shift in the Jhb 25-exposed PMNL with that of the D1317-exposed PMNL, showed a 38% reduction in the median channel shift of the D1317-exposed PMNL relative to the Jhb 25-exposed PMNL. This reduction was significant ($p < 0.05$). The results are shown in Fig. 3.3B.

3.1.2.2 The effect of heat inactivation of MV on the ability of MV-exposed PMNL to phagocytose bacteria

All three MV strains were inactivated by exposure to a temperature of 56^0 C for 1 hour, following which PMNL were exposed to each strain for 1 hour, and phagocytosis then measured.

Exposure of PMNL to heat-inactivated forms of all three MV strains, was accompanied by small increases in the percentage of PMNL that performed phagocytosis. These increases were, however, not enough to restore the percentage of fluorescent cells to that found for the uninfected control (Fig. 3.4A). The Schwartz-HI-exposed PMNL showed an increase of 23% relative to its untreated form ($p < 0.05$), while the apparent increase in the Jhb 25-HI-exposed PMNL relative to its untreated form was not significant ($p > 0.05$). The increase was greater by 43% in the D1317-HI-exposed PMNL when compared to its untreated form ($p < 0.05$). See Fig. 3.4A.

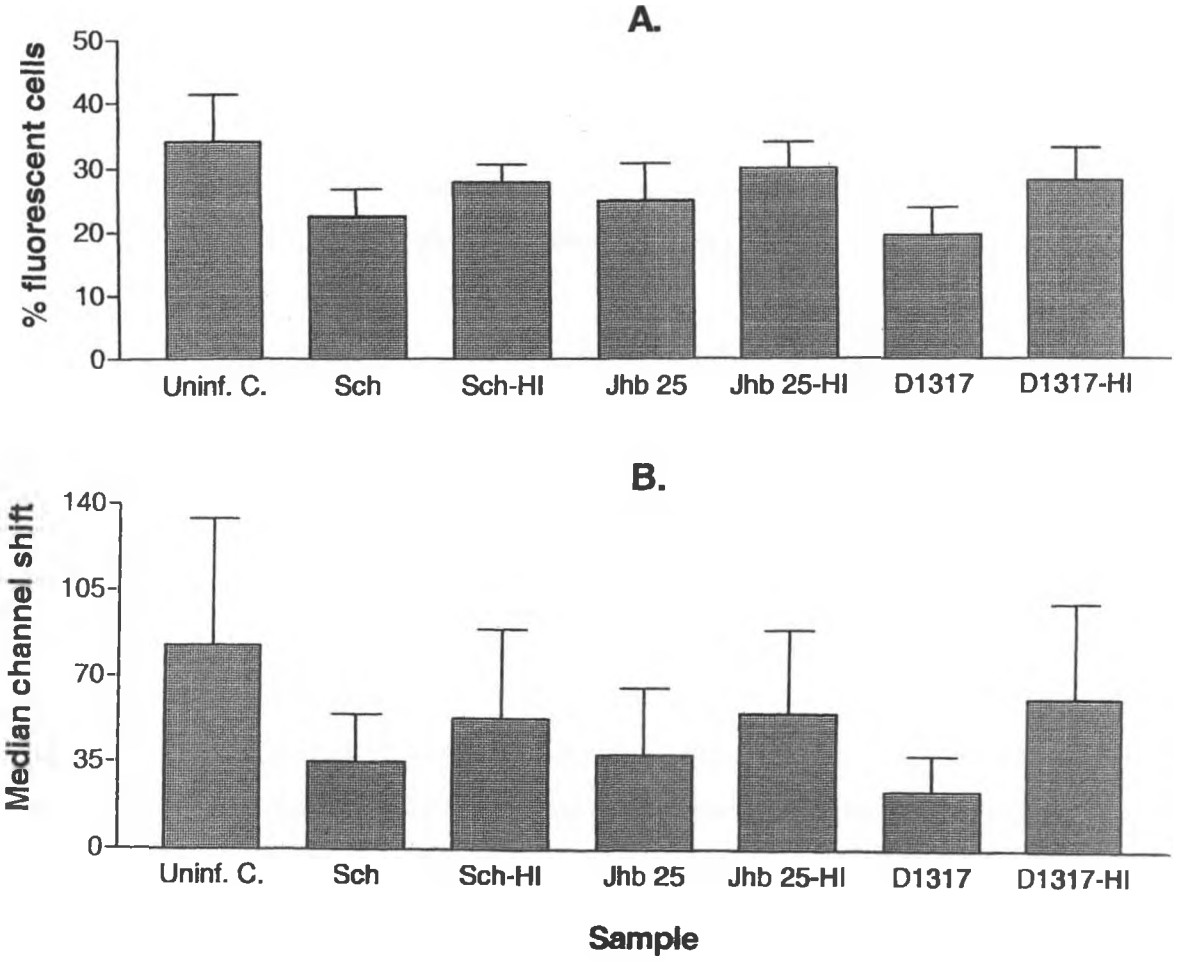


Figure 3.4 : *Phagocytosis results for PMNL exposed to MV and HI-MV strains, as well as the uninfected control, are shown. A. Percentage fluorescent cells. B. Fluorescence intensity (median channel shift).*

Heat inactivation of MV was accompanied by an increase in the number of ingested bacteria per individual cell as seen by increases in fluorescence intensity (median channel shift) of all PMNL exposed to heat-inactivated forms of the three MV strains (Fig. 3.4B). However, this still fell short of restoring phagocytosis to the level of the uninfected control ($p < 0.05$). The reversal occurred to the greatest extent in the D1317-HI-exposed PMNL, which was almost 3 times higher than its untreated form (D1317) ($p < 0.05$). The heat-inactivated forms of Jhb 25 (Jhb 25-HI) and Schwartz (Schwartz-HI) showed increases of 45% and 53% respectively in median channel shift when compared to their untreated forms ($p > 0.05$).

3.1.3 Oxidative burst

The effect of MV on PMNL oxidative burst was quantitatively determined using the Bursttest kit. PMNL were isolated from whole blood obtained from 8 healthy individuals. The percentage of cells having produced reactive oxygen intermediates (ROI) and their fluorescence intensity (median channel shift), were analysed by flow cytometry.

3.1.3.1 PMNL exposure to MV strains and their ability to produce reactive oxygen intermediates

The percentage of PMNL that produced reactive oxygen radicals when induced by *E. coli* was less in all MV-exposed samples when compared to the uninfected control (Fig. 3.5 A). The reduction occurred to the greatest extent in the D1317-exposed PMNL which showed a decrease of 41% in the percentage of cells having produced reactive oxygen radicals, relative to the uninfected control ($p < 0.05$). Both the Schwartz- and the Jhb 25-exposed PMNL showed a reduction of 35% in the percentage of cells having produced reactive oxygen radicals ($p < 0.05$) (see Fig. 3.5 A). There were no significant differences between the different MV strains in their ability to reduce ROI production. Analysis of the fluorescence intensity as determined by the median channel shifts showed the same trends to that found for proportions of fluorescing cells (Fig. 3.5 B), but with all reductions due to exposure to MV found not to be statistically significant ($p > 0.05$). Inter-strain comparison of median channel shifts of the three MV-exposed PMNL samples revealed no significant differences ($p > 0.05$).

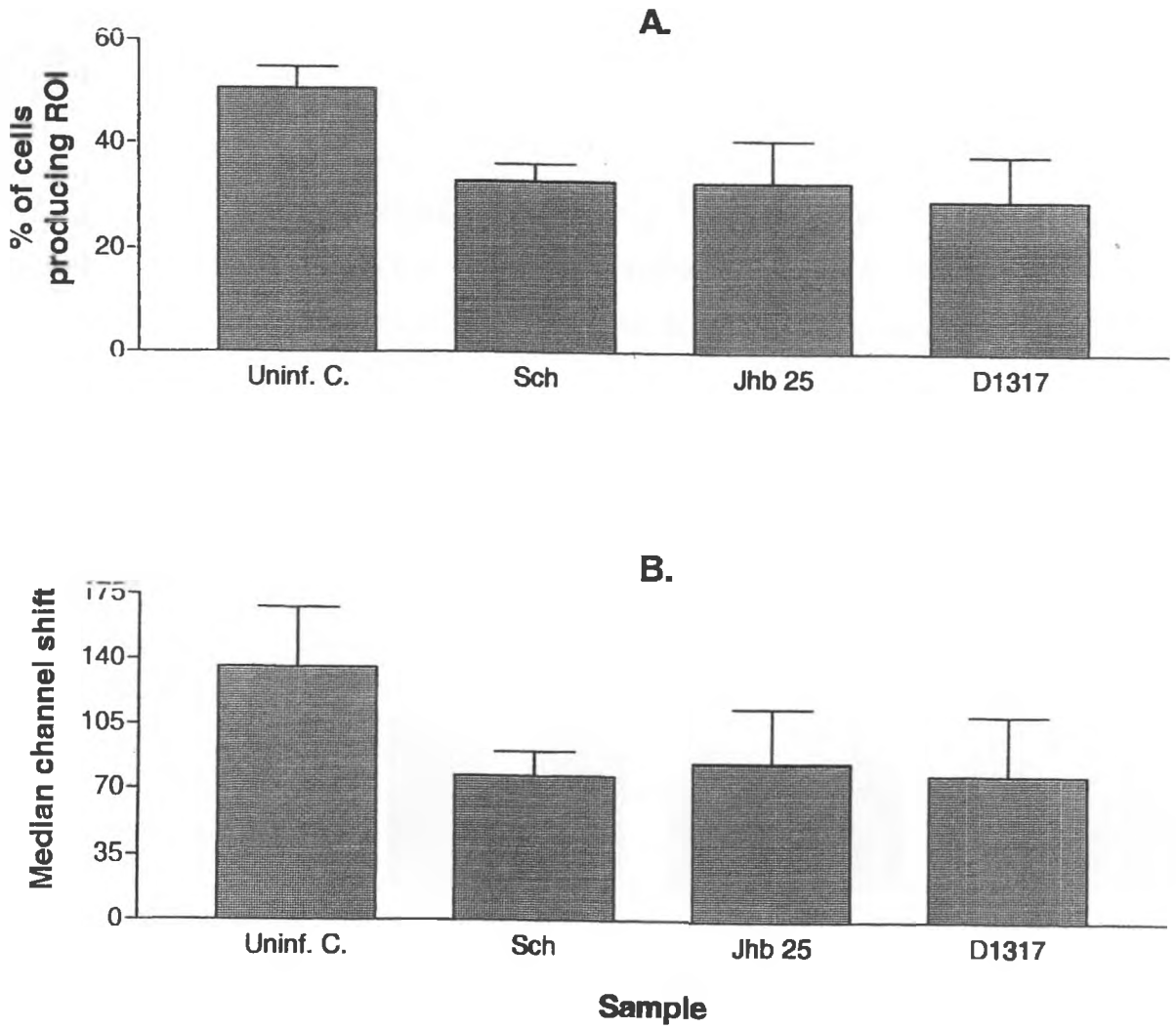


Figure 3.5 : *Oxidative burst results for uninfected PMNL and those of PMNL exposed to MV strains. A. Percentage of cells producing ROI. B. Fluorescence intensity (median channel shift).*

3.1.3.2 The effect of heat inactivation of MV on the ability of MV-exposed PMNL to produce ROI

As with phagocytosis, a reversal of the trends was observed in PMNL exposed to the heat-inactivated forms of the three MV strains. These reversals were apparent mainly in the median channel shift measurements and not in the proportions of PMNL capable of producing reactive oxygen radicals (see Figs. 3.6 A and 3.6 B).

The median channel shift (fluorescence intensity) observed in PMNL exposed to the above MV heat-inactivated strains, tended to be increased relative to those exposed to MV strains not heat-inactivated. The Schwartz-HI-exposed cells showed an increase of 38%, the Jhb 25-HI-exposed PMNL an increase of 31% and D1317-HI-exposed PMNL increase of 15% in the median channel shift compared to their respective untreated forms. Once again, the differences in the number of cells having produced reactive oxygen intermediates, as well as the median channel shifts between the three heat-inactivated MV-exposed PMNL were very small and statistically insignificant ($p > 0.05$).

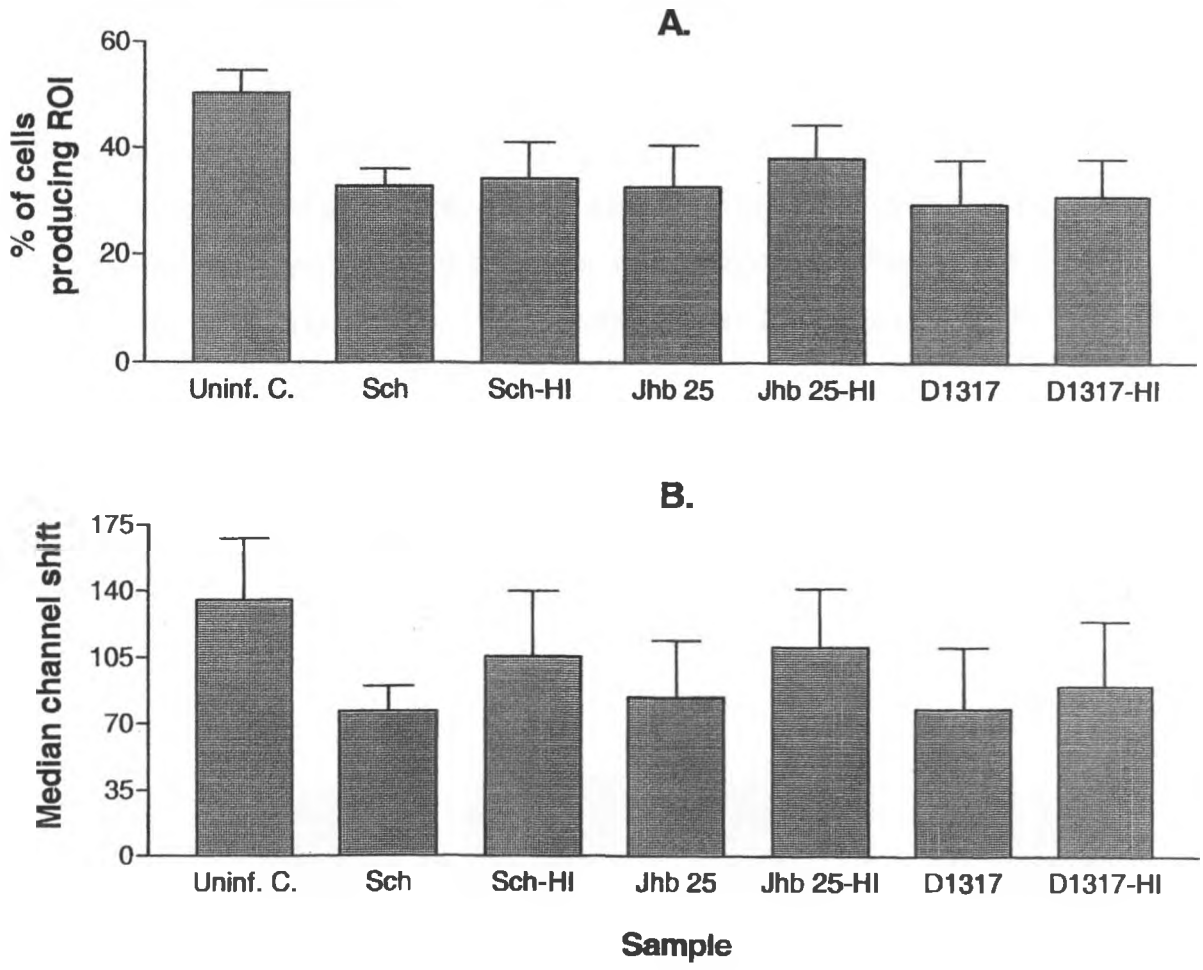


Figure 3.6 : *Oxidative burst results for PMNL exposed to both the MV and HI-MV strains, and the uninfected control. A. Percentage of PMNL having produced ROI. B. Fluorescence intensity (median channel shift).*

3.1.4 Degranulation

The ability of PMNL exposed to the three MV strains, as well as their heat-inactivated forms, to undergo degranulation in the absence and in the presence of IL-8, was assessed using blood obtained from 7 healthy individuals. The results are shown Figs. 3.7, 3.8, 3.9, 3.10, 3.11 and 3.12.

3.1.4.1 PMNL exposure to MV strains and the induction of degranulation

(a) MV induction of the release of β-glucuronidase from PMNL

Before determining if MV could alter degranulation in response to a potent chemokine such as IL-8, it was important to determine if MV alone had the ability to directly affect degranulation of PMNL. PMNL exposed to the different MV strains, showed no differences in the percentage of β-glucuronidase enzyme released from cells when compared to that released by uninfected control PMNL (Fig. 3.7) ($p > 0.05$). Therefore the different MV strains did not have the ability to directly induce degranulation of PMNL.

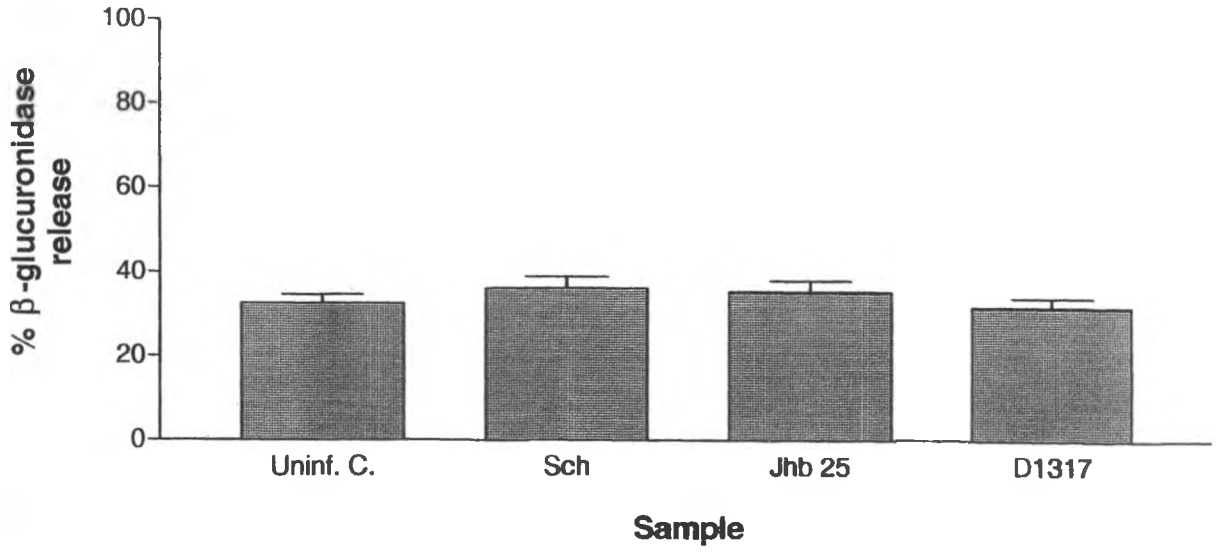


Figure 3.7 : *Degranulation : Spontaneous and MV-induced β-glucuronidase release.*

(b) Interleukin-8 (IL-8) induced release of β-glucuronidase enzyme

It was next questioned whether MV had the ability to alter degranulation in response to a potent agonist, IL-8.Overall, β-glucuronidase release observed in all samples was higher, as would be expected, than that observed in the same samples exposed to a lower IL-8 concentration. Although the MV-exposed PMNL, with the exception of the D1317-exposed PMNL showed a higher percentage enzyme release relative to the uninfected control, inter-strain comparisons showed small and insignificant differences in percentage enzyme release ($p > 0.05$). Therefore, in addition to not directly affecting degranulation, MV strains also had no effect on the ability of IL-8 to adequately induce degranulation.

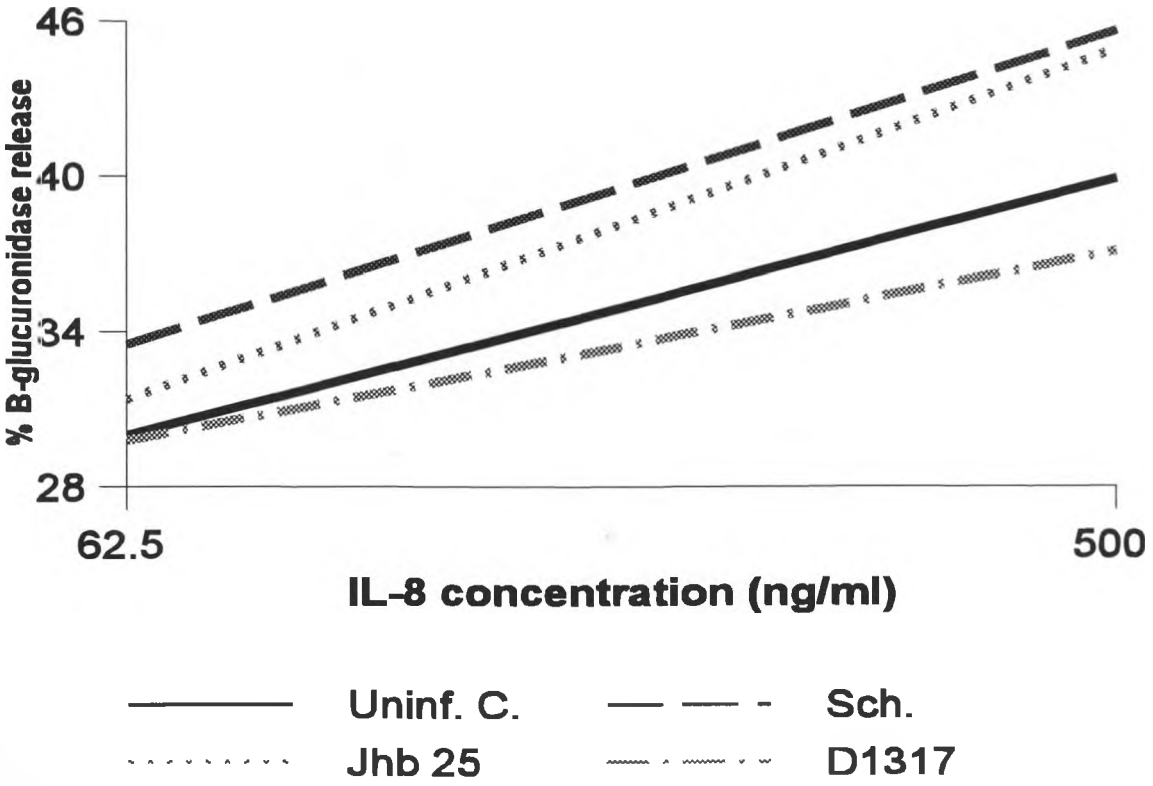


Figure 3.8 : *Percentage enzyme release in the uninfected control and MV-exposed PMNL at a low and high IL-8 concentration.*

3.1.4.2 PMNL exposure to heat-inactivated MV strains and the induction of degranulation

(a) Induction of degranulation by heat-inactivated MV strains

Although, on the basis of the aforementioned findings it would seem unlikely that heat inactivation of the MV strains would have any effect, the HI forms were nonetheless tested. In the absence of IL-8, the percentage enzyme release induced by the heat-inactivated forms of each of the three MV strains was in some instances raised compared to the corresponding untreated form. However, these increases were very small and all statistically insignificant ($p > 0.05$). See Fig. 3.9.

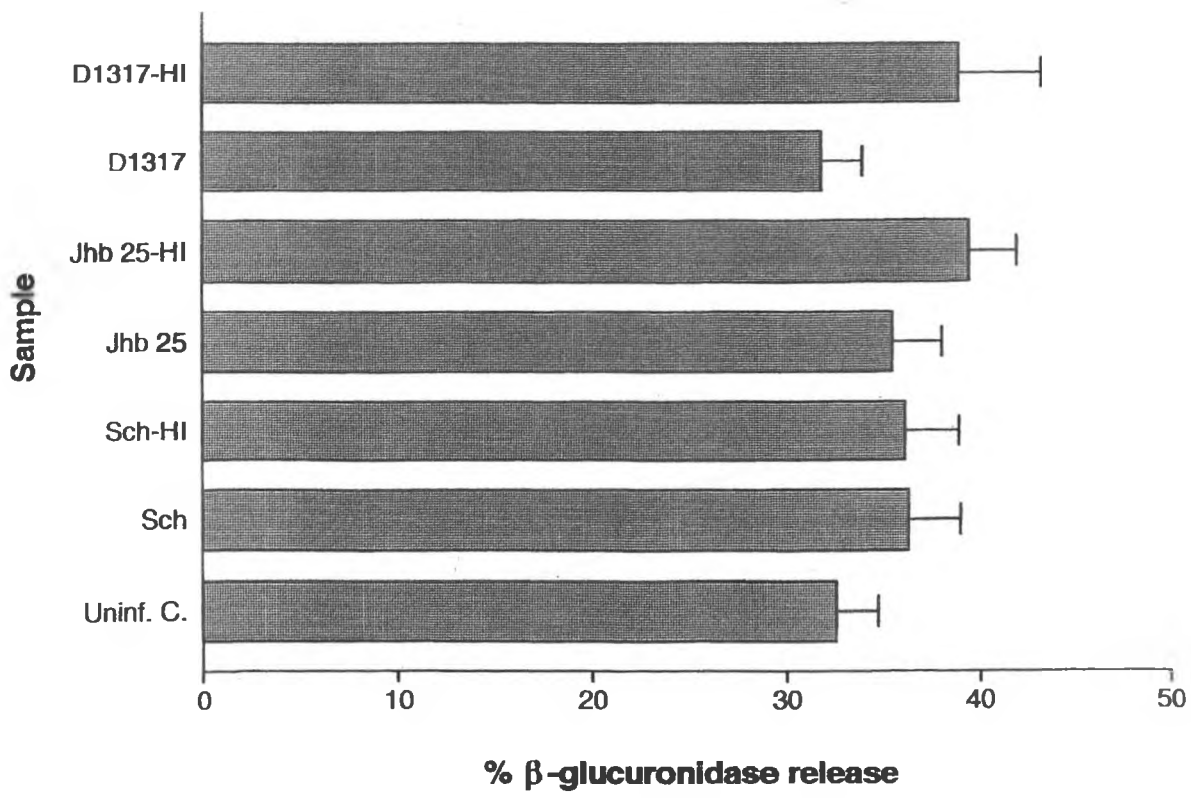


Figure 3.9 : *Comparison of percentage enzyme release between the uninfected control, MV- and HI-MV-exposed PMNL.*

(b) IL-8 induced release of β-glucuronidase

(i) Schwartz and Schwartz-HI

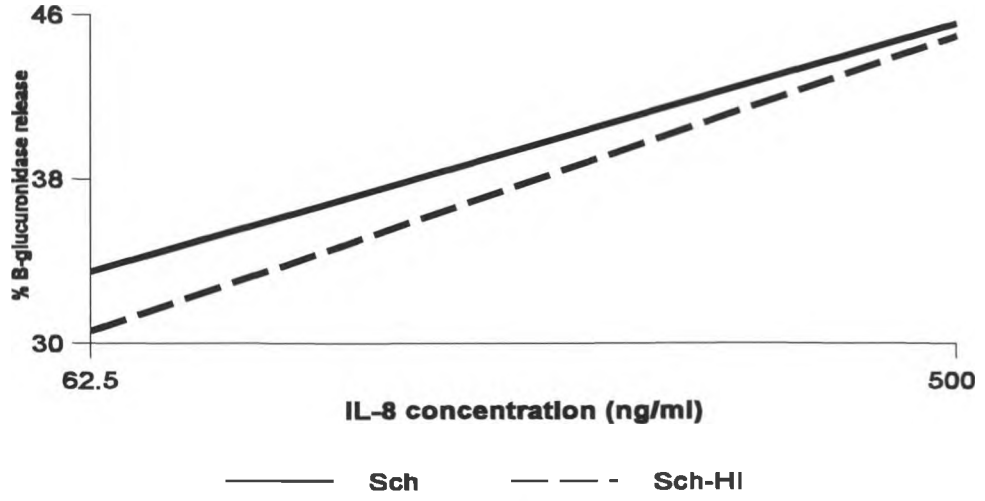


Figure 3.10 : *Comparison of percentage β-glucuronidase release by PMNL exposed to the Schwartz and Schwartz-HI MV strains.*

(ii) Jhb 25 and Jhb 25-HI

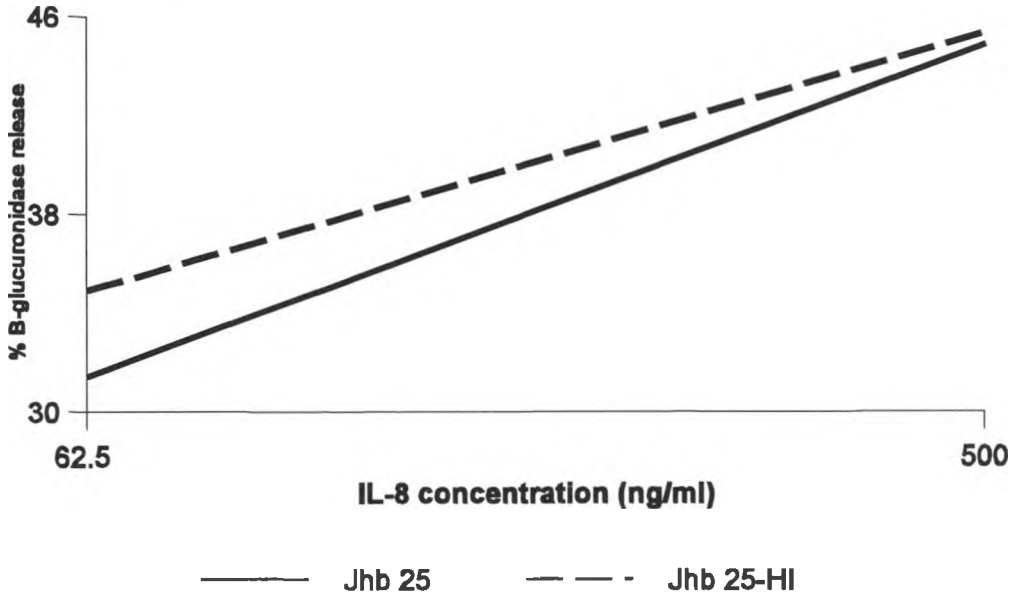


Figure 3.11 : *Comparison of percentage β-glucuronidase release by PMNL exposed to the Jhb 25 and Jhb 25-HI MV strains.*

(iii) D1317 and D1317-HI

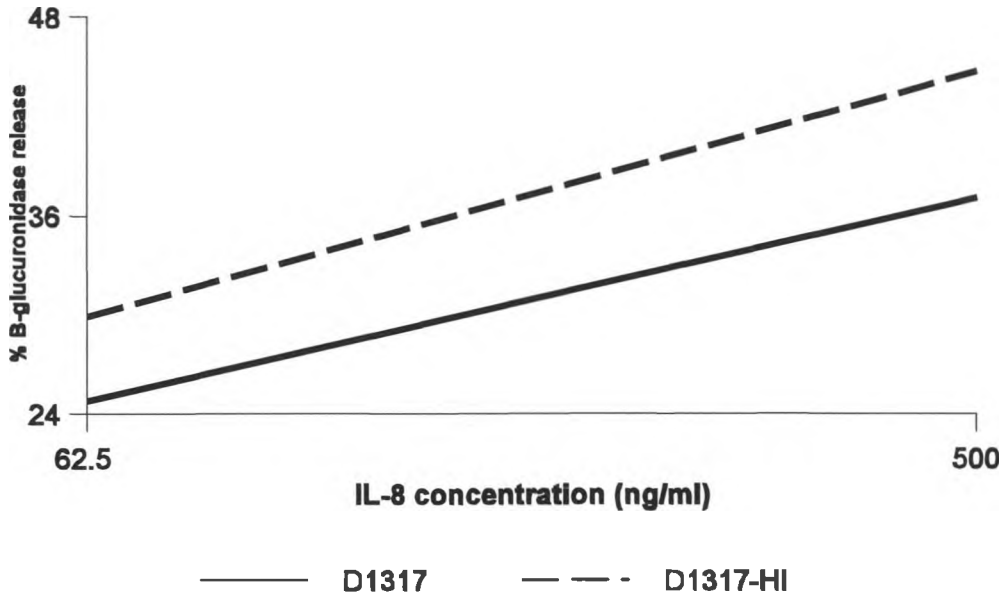


Figure 3.12 : *Comparison of percentage β-glucuronidase release by PMNL exposed to the D1317 and D1317-HI MV strains.*

All HI forms of MV showed no significant effects on degranulation in response to IL-8 (tested at concentrations 62.5 and 500ng/ml) in relation to their non-HI counterparts ($p > 0.05$).

3.2 MV AND INDUCTION OF APOPTOSIS

A Cell Death Detection ELISA^{PLUS} kit was used to quantify histone-associated DNA fragments (mono- and oligonucleosomes) present in the cytoplasm of cells undergoing apoptosis. The ability of MV strains to directly induce apoptosis in PMNL was determined by first exposing PMNL from 6 healthy blood donors to each of the strains for 1 hour and then measuring the extent of apoptosis.

3.2.1 Exposure of PMNL to different MV strains and the induction of apoptosis

Apoptosis was induced in PMNL by all three MV strains ($p < 0.05$). As depicted in Fig. 3.13, this

occurred to the greatest extent (90%) with the wild-type D1317 strain, which in turn was greater than that induced by both vaccine (28%), and vaccine-like strains (54%) ($p < 0.05$). Although there was a trend towards increased apoptosis when PMNL were exposed to the Jhb 25 strain as opposed to the Schwartz strain, this was not found to be significant ($p > 0.05$).

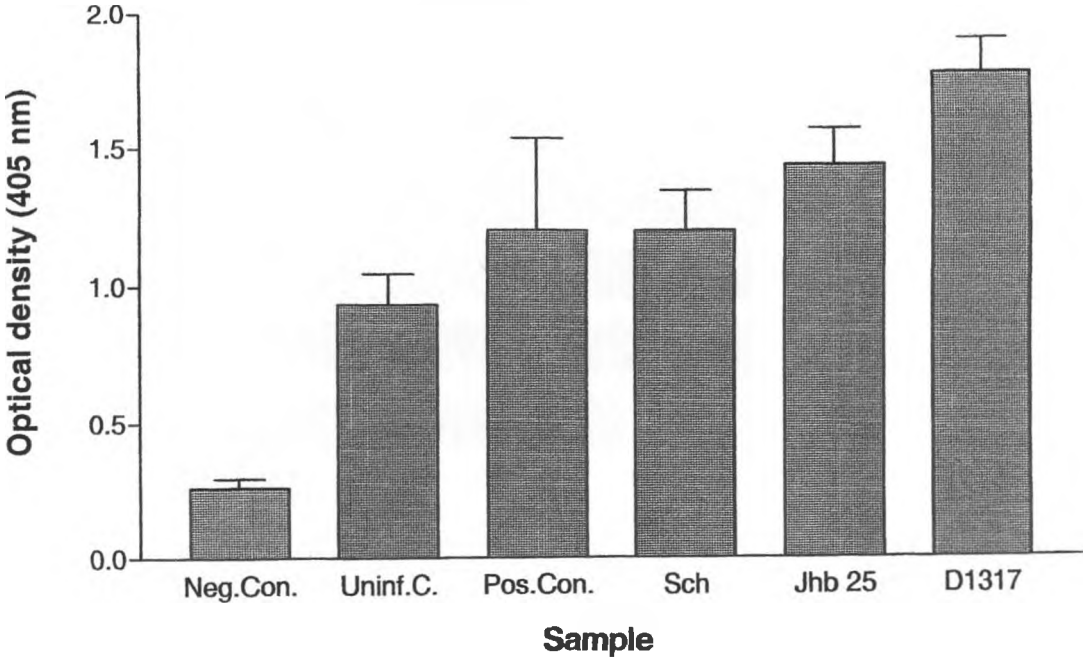


Figure 3.13 : ***Apoptosis measured in PMNL exposed to the three MV strains, the negative control (Neg. Con.), uninfected control (Uninf. C.), as well as the positive control (Pos. Con).***

D1317-exposed PMNL showed an increase of 49% ($p < 0.05$) and 24% ($p < 0.05$) when compared to the Schwartz- and Jhb 25- exposed PMNL, respectively (Fig. 3.13). Apoptosis was also observed in the uninfected controls, the reason for that being that PMNL are terminally differentiated cells, and once isolated from whole blood they begin to undergo apoptosis at a slow rate.

3.2.2 Heat inactivation of MV strains and induction of apoptosis

Heat inactivation of MV strains only significantly reduced apoptosis induced by the wild-type strain D1317 by 29%, this still being 35% above that of the uninfected control ($p < 0.05$) (see

Fig. 3.14). Heat inactivation of MV, however, had no significant effect on apoptosis induced by either the Schwartz (vaccine) strain or the Jhb 25 (vaccine-like) strains ($p > 0.05$).

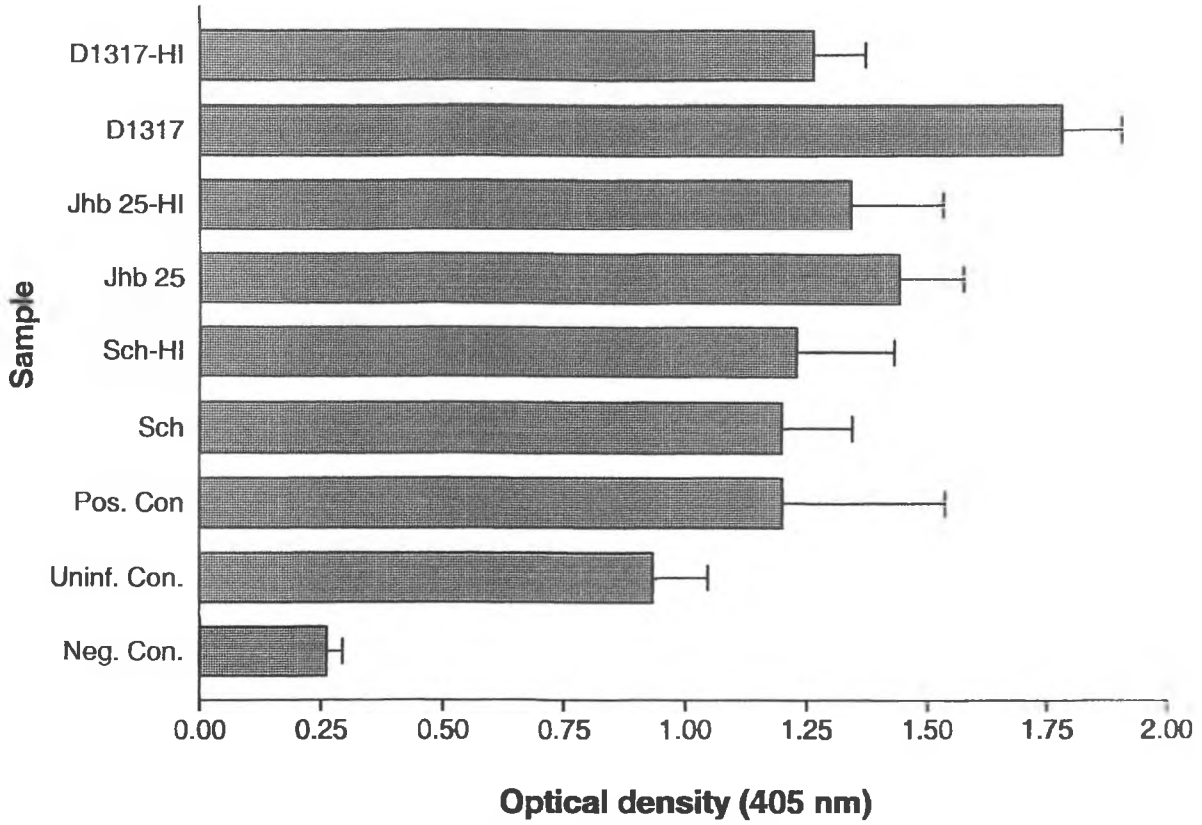


Figure 3.14 : ***Apoptosis in PMNL exposed to MV and HI-MV strains, uninfected control, negative control, and positive control.***

3.3 LIGATION OF THE MV RECEPTOR BY ANTI-CD46 ANTIBODY

CD46 functions as the cellular receptor for MV. As MV is not known to productively infect PMNL, and exposure of PMNL to MV in the above experiments was only for 1 hour, defects in various PMNL functions described are likely to be due to an early event in the MV-cell interaction. In order to establish if the binding of MV to the receptor could be the event leading to the reduction in the various PMNL functions described, it was questioned whether ligation of the CD46 receptor by an antibody that specifically binds this receptor, could in a similar way reduce specific PMNL functions. The ability of CD46 antibody to affect IL-8 induced chemotaxis and to induce apoptosis was therefore studied. The results are shown in Figs. 3.15 and 3.16.

3.3.1 Chemotaxis

Chemotaxis was inhibited by CD46 antibody in a dose-dependent manner. A reduction of 5% in the chemotactic index was found when a low concentration of anti-CD46 (5 ug/ml) was added to cells. The reduction was more pronounced in those PMNL exposed to a higher antibody concentration (25 ug/ml), and was 24% less than that recorded in PMNL exposed to a low CD46 antibody concentration ($p < 0.05$). These reductions from normal in chemotactic indices due to both the low and high antibody concentrations were significant ($p < 0.05$).

Exposure of PMNL to different concentrations of the control isotype, IgG_1, was accompanied by an increase in the chemotactic index relative to the uninfected control. For PMNL exposed to a low concentration of IgG_1 (5 ug/ml), an increase of 11% in chemotactic index, relative to the uninfected control, was observed (Fig. 3.15). In those PMNL exposed to a higher IgG_1 concentration (25 ug/ml), the increase in chemotactic index was 32% higher than that recorded

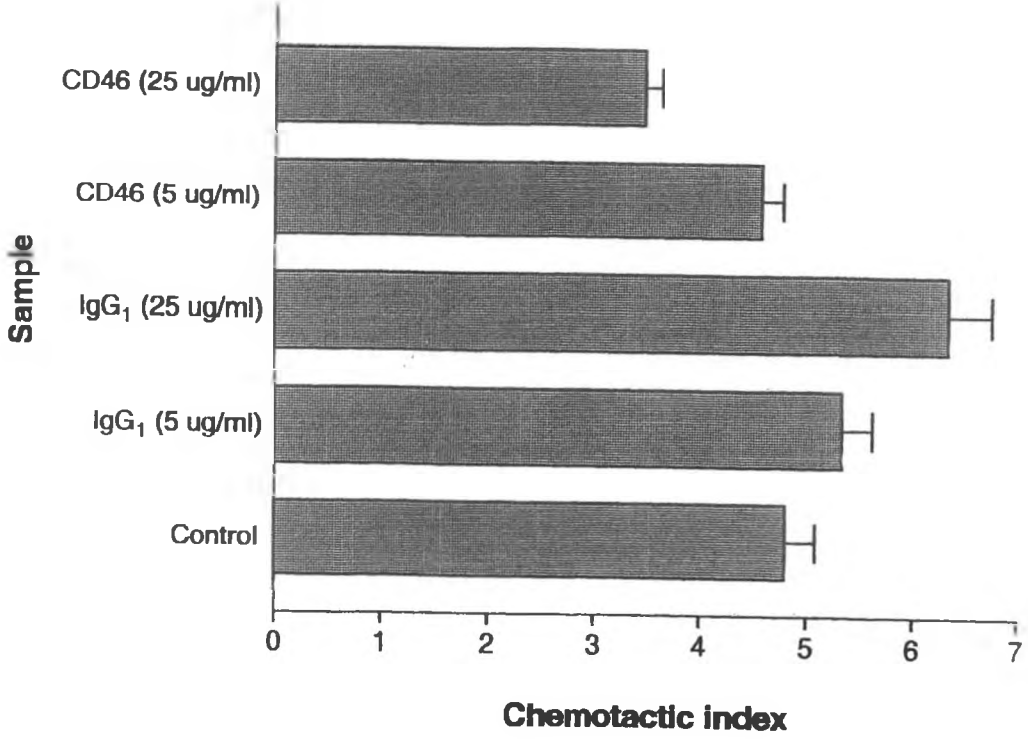


Figure 3.15 : *Chemotactic indices of untreated PMNL (control) and those of PMNL exposed to both the high and low concentrations of the CD46 and IgG_1 antibodies.*

for the uninfected control. Both increases were significant ($p < 0.05$). The chemotactic index recorded for those PMNL exposed to a higher IgG_1 antibody, was significantly raised relative to that recorded in those PMNL exposed to a lower concentration ($p < 0.05$). Therefore an increase in the concentration of the control isotype antibody, IgG_1, was accompanied by an increase in the chemotactic index.

In summary, exposure to the CD46 antibody led to an inhibition of chemotaxis as seen by the reduction of in the chemotactic index with an increase in dose. On the other hand, exposure of PMNL to the isotype control IgG_1 antibody had an enhancing effect on chemotaxis of PMNL as shown by an increase in the chemotactic index as the dose was increased. These latter findings strongly suggest that the effects on chemotaxis of the CD46 antibody binding to PMNL are not in anyway due to non-specific binding of the CD46 antibody to the F_C receptors. Furthermore, binding of F_C receptors by IgG_1 molecules in their own right elicit effects quite opposite to that of the CD46 antibody on chemotaxis.

3.3.2 Apoptosis

After exposure of PMNL to two concentrations of the anti-CD46 antibody (5 ug/ml and 25 ug/ml) and two concentrations of the isotype control antibody, IgG_1, (5 ug/ml and 25 ug/ml), for a period of 1 hour, apoptosis was determined by measuring the optical densities of the samples in the apoptosis assay (ELISA). This measurement was carried out in order to determine whether occupation of the CD46 receptors by these antibodies would have any effect on apoptosis.

Exposure of PMNL to both concentrations of the CD46 antibody, was accompanied by an increase in optical density relative to the uninfected control ($p < 0.05$). The greatest increase in apoptosis was recorded in those PMNL exposed to the high CD46 antibody concentration (25 ug/ml). An increase of 220% relative to the uninfected control was observed. At the low CD46 antibody concentration of 5 ug/ml, the optical density observed was lower than that recorded at the high antibody concentration, but was nearly twice that of the uninfected control. The difference in optical density between PMNL exposed to 5 ug/ml and 25 ug/ml of the CD46 antibody, was significant ($p = 0.05$).

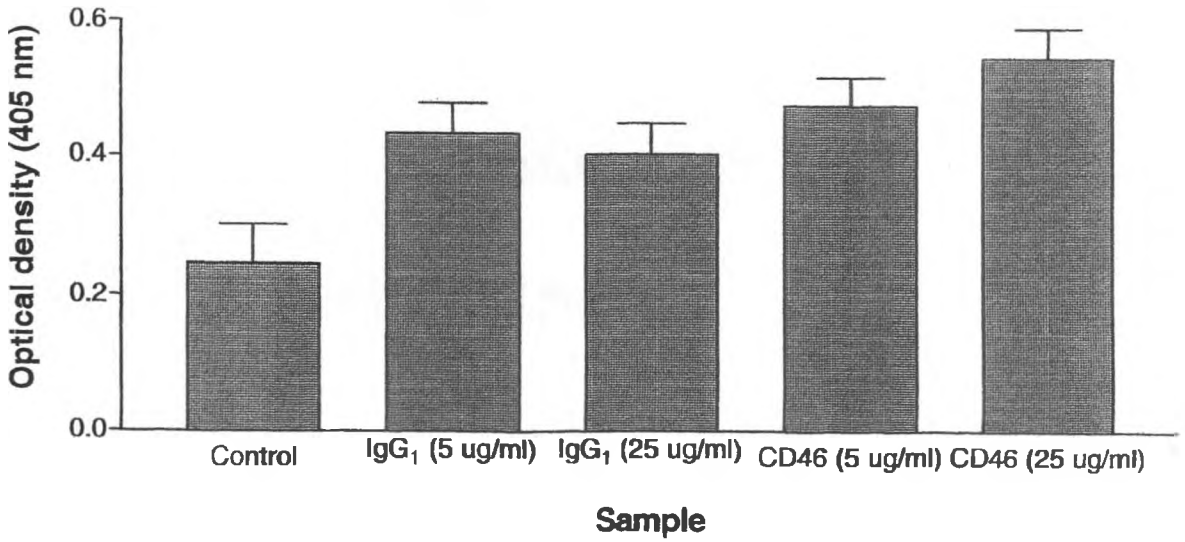


Figure 3.16 : *Optical densities from PMNL samples exposed to the two concentrations of the CD46 and IgG_1 antibodies are compared to that of untreated PMNL (control).*

The trend was similar for IgG_1 antibody-exposed PMNL to that observed for the anti-CD46-exposed PMNL. PMNL exposure to the isotype control antibody, IgG_1, resulted in an increase in apoptosis, as seen by increases in the optical densities of samples exposed to the two concentrations of this antibody (Fig. 3.16). The optical density recorded in PMNL exposed to a low IgG_1 concentration (5 ug/ml) was 75% higher than that recorded in the uninfected control ($p < 0.05$). As the concentration of the IgG_1 antibody was raised from 5 ug/ml to 25 ug/ml, the optical density dropped, but still remained elevated (63% higher) compared to the uninfected control ($p < 0.05$). Therefore, apoptosis was induced to some extent by exposure to IgG_1 antibody but not to the extent of that induced by anti-CD46 antibody.

CHAPTER 4

DISCUSSION

4.1 MV AND PMNL FUNCTIONS

4.1.1 Chemotaxis

Chemotaxis, a process of directed movement of cells along a gradient of increasing concentration of the attracting molecule (referred to as a chemoattractant), is a property that cells of the immune system use to migrate to the site of infection where the concentration of chemotactic factors is highest (i.e. the epicentre of the inflammatory process). PMNL are the first cells of the immune system to migrate to the site of microbial invasion, and are the principal cells involved in the immune response to invasion of the host by microbes, particularly bacteria and fungi.

Exposure of PMNL to all three MV strains used in this study, viz :- Schwartz (vaccine strain), Jhb 25 (vaccine-like strain) and D1317 (wild-type strain), resulted in significant reductions in PMNL chemotaxis in response to a chemoattractant, IL-8. The wild-type strain D1317 had the greatest impact in this regard. However, when compared to other strains, it was found to be significantly different only to the Jhb 25 exposed PMNL.

A slight recovery of function was observed when PMNL were exposed to MV strains which had been inactivated by heating at 56^0 C for 1 hour. This recovery failed to bring about complete restoration of the chemotactic function of PMNL, and as such, the chemotactic indices remained significantly reduced when compared to the uninfected control. Clearly, the movement of PMNL in response to a chemoattractant, IL-8, was reduced by exposure of these cells to both the MV and heat-inactivated MV strains.

4.1.2 Phagocytosis

A key component of any host defence system is the ability to ingest and destroy offending microorganisms. In the phagocytic process, PMNL are capable of ingesting more than one microorganism at once. In situations where large numbers of PMNL are involved in this process, an abscess filled with pus (dead or dying PMNL) may form.

The phagocytic ability of PMNL was clearly compromised by exposure of these cells to all MV strains. Both the proportion of cells phagocytosing bacteria (*E. coli*), and the number of bacteria within the cells were significantly reduced. As with chemotaxis, the wild-type MV strain, D1317 had the greatest impact on the phagocytic function of PMNL where both the proportion of cells performing phagocytosis, defined as percentage fluorescent cells, and the number of bacteria ingested by each PMNL, defined as the median channel shift, were the lowest recorded. The fact that the number of bacteria phagocytosed by PMNL exposed to the wild-type strain D1317 were reduced by 71% when compared to those ingested by PMNL in the uninfected control, is testimony to the fact that the wild-type strain D1317 was the most potent inhibitor of phagocytosis, of the three MV strains used. It was also interesting to note that PMNL exposed to all MV strains were only capable of phagocytosing less than half the number of bacteria that PMNL in the uninfected control could ingest.

Virus inactivation, through heating, brought about some improvement in PMNL phagocytic ability. Both the percentage of cells ingesting bacteria and the number of bacteria ingested in each cell rose when PMNL were exposed to heat-inactivated MV strains. As in the case of untreated MV strains, the trend observed with PMNL exposed to heat-inactivated MV strains was the same in both the percentage of cells phagocytosing bacteria and the fluorescence intensity. As with chemotaxis, recovery of function was observed in PMNL exposed to heat-inactivated MV strains, but complete restoration of function was not achieved. Both the percentage of PMNL ingesting bacteria and the number of phagocytosed bacteria within PMNL exposed to heat-inactivated MV strains, remained significantly reduced relative to the uninfected control.

4.1.3 Oxidative burst

This is a mechanism through which phagocytosed microorganisms can be killed. It is an oxygen-dependent mechanism and is also called the respiratory burst. It involves the production of reactive oxygen intermediates which are highly toxic to ingested microorganisms.

As with chemotaxis and phagocytosis, this PMNL function was negatively affected by exposure of PMNL to the three MV and the three heat-inactivated MV strains. Significant reductions in both the percentage of cells producing reactive oxygen intermediates (ROI), measured as the percentage of fluorescent cells, and their fluorescence intensity, measured as the median channel shift were observed. The trends were the same for both the percentage of fluorescent cells and the median channel shifts. While oxidative burst was significantly reduced in PMNL exposed to all three MV strains relative to the uninfected control, an inter-strain comparison of percentage fluorescent cells and median channel shift of PMNL exposed to all three MV strains revealed no significant differences.

Heat inactivation of MV strains and subsequent exposure of PMNL to these strains had a negligible impact on the percentage of fluorescent cells and fluorescence intensity. As observed for PMNL functions of chemotaxis and phagocytosis, exposure of PMNL to heat-inactivated MV strains, although accompanied by an improvement in oxidative burst, failed to bring about complete restoration of the oxidative burst function of PMNL not exposed to MV.

4.1.4 Degranulation

This is the other of the two mechanisms by which phagocytosed microbes can be killed, and unlike oxidative burst is an oxygen-independent mechanism. It involves the release of microbicidal enzymes such as lysozyme and cathepsins into an intracellular vacuole, the phagosome, and the digestion of the ingested microbe by these enzymes.

Percentage enzyme release, in the absence of IL-8, in PMNL exposed to all three MV, was similar to that observed for uninfected PMNL. Inter-strain comparisons and comparisons with

the uninfected control in IL-8 induced degranulation assays revealed no differences. Degranulation in all PMNL preparations (untreated and MV-infected) was raised when measured in the presence of a high IL-8 concentration (500 ng/ml), as compared to a low IL-8 concentration. MV therefore did not alter the expected dose-dependent effects of IL-8 on PMNL degranulation.

While PMNL functions of chemotaxis, phagocytosis and oxidative burst were severely compromised by exposure of these cells to MV and heat-inactivated MV strains, the PMNL function of degranulation appears not to have been affected by exposure to the MV strains.

4.2 MV AND INDUCTION OF APOPTOSIS

Apoptosis, also called programmed cell death, is a genetically controlled pre-programmed event, through which unwanted cells are eliminated during development. Several microorganisms are known to induce apoptosis.

In this study, it was found that all three MV strains considered, significantly induced apoptosis in PMNL. Of the three MV strains, the wild-type strain, D1317, was the most potent inducer of apoptosis in PMNL. Apoptosis due to this strain was significantly raised when compared to that in the uninfected control, the Schwartz- and the Jhb 25-exposed PMNL. No significant difference in apoptosis was observed when the Schwartz-exposed PMNL were compared to the Jhb 25-exposed PMNL.

Heat-inactivation of MV strains did not have any appreciable effect on apoptosis induced by any of the strains, relative to the uninfected control. Apoptosis in PMNL exposed to heat-inactivated forms of all three MV strains, though slightly reduced relative to their untreated forms, were still high when compared to that of the uninfected control. The only significant reduction in apoptosis was observed when the D1317-exposed PMNL were compared to the D1317-HI-exposed PMNL. Therefore, in addition to effects of MV on different PMNL functions, MV also had the ability to directly induce apoptosis of these cells.

4.3 LIGATION OF THE MV RECEPTOR BY ANTI-CD46 ANTIBODY

The membrane co-factor CD46 functions as a receptor for MV. This receptor was ligated by anti-CD46 antibody (an antibody that specifically binds this receptor) for the purpose of establishing whether binding of MV to this receptor could be the event leading to the suppression of PMNL functions described. The ability of CD46 antibody, through binding to the CD46 receptor, to affect IL-8-induced chemotaxis and to induce apoptosis of PMNL was therefore chosen for study.

4.3.1 Chemotaxis

PMNL exposure to the CD46 antibody was accompanied by a reduction in chemotaxis. This reduction occurred in a dose-dependent manner, i.e. a lower chemotactic index was observed in PMNL exposed to the higher CD46 antibody concentration. The implication here is that as the CD46 antibody concentration is raised, this leads to an increased occupation of the CD46 receptors on PMNL by this antibody, and this negatively affects PMNL chemotaxis as seen by the reduction in the chemotactic index.

Interestingly, the opposite results were observed when PMNL were exposed to the two concentrations of the isotype control antibody, IgG_1. This antibody was included to ensure that CD46 antibody effects were specific and not due to non-specific binding of antibody. Even at a low IgG_1 antibody concentration, the chemotactic index of PMNL exposed to this antibody was elevated when compared to that in the uninfected control. As the isotype control antibody concentration was raised, the chemotactic index increased. Therefore, this antibody rather than having no effect appeared to enhance IL-8 induced chemotaxis. These results are suggestive of a role for ligation of F_c receptors (by anti IgG_1 antibody) of PMNL in chemotaxis induced by IL-8. PMNL are known to have large numbers of F_c receptors on their membranes (Abramson and Wheeler, 1994).

4.3.2 Apoptosis

Exposure of PMNL to the CD46 antibody significantly increased apoptosis in these cells with increasing anti-CD46 concentration. The trend observed was the opposite of that observed with chemotaxis where the reduction occurred in dose-dependent manner. It was also noted that at a low CD46 antibody concentration, apoptosis was still significantly elevated relative to the uninfected control.

At a low isotype control IgG_1 concentration, PMNL apoptosis was significantly increased. Raising the concentration of the isotype control antibody was accompanied by a reduction in apoptosis. As seen with chemotaxis, this antibody by binding F_c receptors also affects PMNL apoptosis. The trend observed in the IgG_1 antibody-exposed PMNL was the opposite of that noticed in the CD46 antibody-exposed PMNL. While increasing the IgG_1 antibody concentration led to a decrease in apoptosis (although both still significantly increased relative to the control), increasing the CD46 antibody concentration was accompanied by an increase in apoptosis.

The effects of anti-CD46 antibody, by specifically binding CD46, appear to mimic those of the different MV strains on both PMNL chemotaxis and apoptosis, suggesting that these effects are mediated at the level of virus binding to CD46 on PMNL. These results would further suggest that increased occupation of MV receptors on PMNL by increased doses of MV would likely exacerbate the negative effects on the cell functions studied, as well as increase the rate of apoptosis.

CHAPTER 5

CONCLUSION

The role of PMNL in controlling infection by viruses within the host is often minimal compared to that of mononuclear cells. However, PMNL are the principal cells involved in the immune response to invasion of the host by bacteria and fungi. They provide the first line of defence against invasion of humans by bacterial and fungal microorganisms. Therefore any virus-induced PMNL dysfunction, resulting in alteration of any of the PMNL functions mentioned in the preceeding chapters (chemotaxis, phagocytosis, degranulation and oxidative burst) could result in a situation were there is decreased movement of PMNL to the site of microbial infection, and lead to depressed bactericidal activity against invading organisms (Abramson and Mills, 1988).

Several viruses have been implicated in the depression of one or more of these PMNL functions. In addition to quantitative defects in PMNL function induced by viruses, qualitative deficiencies of PMNL can also occur in patients with viral infections. In patients who experience a decrease in the number of peripheral blood PMNL to less than 1 000 cells/mm^3, there is an inverse correlation between the absolute number of circulating PMNL and the incidence of bacterial and fungal infections. Patients with qualitative or quantitative PMNL abnormalities are prone to similar infectious complications - recurrent tissue infections due to bacterial or fungal agents that invade from skin or mucous membranes. In both groups of patients, organisms such as *Staphylococcus aureus*, enteric species, and candidal species are among the commonest. While localized infections in neutropenic patients commonly progress to life-threatening or irreversible septicaemia, established infections in patients with disorders of leukocyte function generally are limited or contained within superficial tissues or the reticuloendothelial system (Abramson and Mills, 1988).

Quantitative defects such as neutropenia are common in HIV-1 infected individuals (Jacobson *et al.*, 1997). Other studies conducted report functional defects in PMNL from HIV-1-infected patients, and include defects in phagocytosis (Lazzarin *et al.*, 1986; Shalekoff *et al.*, 1998),

chemotaxis (Valone *et al.*, 1984; Ellis *et al.*, 1988; Meddows-Taylor *et al.*, 1998), bacterial killing (Ellis *et al.*, 1988; Murphy *et al.*, 1988), oxidative burst (Chen *et al.*, 1993; Pitrak *et al.*, 1993; Shalekoff *et al.*, 1998) and degranulation (Meddows-Taylor *et al.*, 1999).

While the role of viruses such as Influenza, Parainfluenza, Cytomegalovirus, Herpes simplex, Varicella zoster, Hepatitis B, HIV and Respiratory syncytial virus, in the suppression of PMNL functions, and the impact that this suppression has on predisposition to secondary microbial infections, have been thoroughly studied, the same is not true for MV. The only study in this regard has been conducted by the Anderson group (Anderson, *et al.*, 1976), who reported depressed PMNL chemotactic activity in children with MV infection. How MV infection results in suppressed PMNL function, directly through interaction of virus and the host cell, or indirectly through MV-induced immune processes, is not known.

This study, therefore, set out to determine whether MV had any suppressive effect on a number of PMNL functions. The direct effect of MV on chemotaxis, phagocytosis, oxidative burst, degranulation and on apoptosis of PMNL was therefore studied. This study reports, for the first time, that direct exposure of PMNL to MV strains, whether wild-type or vaccine, heat-inactivated or not, had an inhibitory effect on several PMNL functions. The PMNL function of degranulation was not affected by exposure to MV strains and their heat-inactivated forms. This study has further revealed that exposure of PMNL to all MV strains used resulted in an increase in the rate at which the cells underwent programmed cell death (apoptosis).

Overall, the wild-type strain, D1317, had the greatest suppressive effect on PMNL function of the three strains tested. Wild-type MV strains have been found to be more virulent than vaccine strains (Lorenz and Albrecht, 1980). MV replication following acute infection is accompanied by the production of large amounts of virus. This will most likely have a greater impact on PMNL function *in vivo*. In general, the level of suppression of PMNL functions was less in the vaccine (Schwartz)-exposed PMNL when compared to the vaccine-like (Jhb 25)-exposed and the wild-type (D1317)-exposed PMNL. It is believed that attenuation of virulence is associated with decreased virus replication in lymphatic tissue (Ono *et al.*, 1970), diminished viraemia (von Binnendijk *et al.*, 1994) and failure to induce pathologic changes in neural tissue (Buynak *et al.*,

1962). It can be seen in this study that even though direct effects due to vaccine strains are evident, because of reduced viral replication due to virus attenuation, the low amount of virus produced is more likely to have less of an effect on PMNL. From the results of this study it is clear that attenuation of MV strains could to a small extent abrogate the PMNL suppression observed with wild-type virus.

The question of virus inactivation, was investigated by subjecting all three MV strains to a temperature of 56^0 C for 1 hour, prior to exposing PMNL. There were slight improvements in PMNL functions relative to non-HI virus, though not adequate (i.e. PMNL functions never improved to the level observed in the uninfected control). Heat-inactivation certainly brought about a slight abrogation or suppression, although this was not enough to completely restore normal function. This suggests that the process of heating could be affecting some protein or protein activity (perhaps enzymatic) involved in MV binding and/or internalization. It may be significant that heat inactivation of the wild type virus significantly decreased apoptosis in wild type MV-exposed PMNL, suggesting that wild type virus may in fact have a more potent enzyme activity that is affected by heat than the more attenuated strains.

In addition to the qualitative defects in PMNL functions observed in this study, the issue of quantitative defects in PMNL should also be considered. MV could be capable of reducing the number of circulating PMNL *in vivo* by inducing apoptosis (i.e. accelerating the rate at which these cells are 'committing suicide'), directly through virus binding to CD46 receptors or indirectly through MV-induced immune processes. Suppression of the above functions and reductions of PMNL in the MV-infected host could be the result of increased apoptosis of these cells. It is a known fact that cells of multinuclear organisms are capable of self-destruction in response to a wide variety of stimuli, by simply activating an intrinsic cell suicide programme within them. This normally happens when these cells are no longer needed or have become seriously damaged, either through radiation or viral infection, among others. This process called apoptosis, is a genetically preprogrammed event which occurs in a controlled manner to eliminate unwanted cells during the course of normal development (Teodoro and Branton, 1977). A variety of stimuli, physiological and non-physiological, among them metabolic disturbances due to chemical insults, virus infections or aberrant growth induced by the activation of oncogenes, can

provoke or accelerate this process (vaux and Strasser, 1996). If this happens, an imbalance can be created between the number of cells produced and those eliminated, i.e. more cells will be destroyed than can be replaced at any one time. As a result a decrease in circulating PMNL can occur, and this can also predispose the host to bacterial and fungal infections.

Apoptosis was significantly raised in all PMNL samples directly exposed to MV strains. The highest apoptotic rate was recorded in the D1317 (wild-type) -exposed PMNL samples. This accelerated PMNL destruction due to virus exposure could have severe consequences for the MV-infected host as this could affect the ability of PMNL to respond appropriately to invading bacteria or fungi, these cells being compromised in terms of function (chemotaxis, phagocytosis, oxidative burst) and perhaps in terms of circulating cell numbers.

Having observed all these defects due to MV, the mechanism involved in this suppression was questioned. As MV is not known to productively infect PMNL, and exposure of PMNL to MV in all the experiments conducted was only for 1 hour, the defects observed in the PMNL functions described are likely due to an early event in the MV-cell interaction. In order to establish if the binding of MV to the receptor could be the event leading to the suppression observed in PMNL functions, it was questioned whether ligation of the MV-specific receptor, CD46, by an antibody that specifically binds this receptor, could in a similar way affect PMNL functions.

As mentioned earlier, the transmembrane protein CD46 is a receptor for MV. It is a member of the cluster of genes that regulate complement activation and protects cells from autologous complement-mediated damage (Liszewski, *et al.*, 1991). CD46 can occur in as many as 14 different isoforms because of alternative splicing of its mRNA (Russel, *et al.*, 1992). All isoforms share, at the N-terminus, a fourfold repeat of the short consensus repeat (SCR) region, a characteristic feature of the regulators of complement activation located close to the plasma membrane and the first part of the transmembrane domain. The isoforms differ in their use of three exons encoding three STP (Ser, Thr, and Pro)- rich regions (called A, B and C) and in three exons encoding the second part of the transmembrane region and the cytoplasmic tail (the two major ones are called CYT 1 and CYT 2). The expression of the various CD46 isoforms varies

from one tissue to another and may potentially govern tissue sensitivity to MV infection (Russel, *et al.*, 1992).

Binding of MV is the primary function of CD46 as a virus receptor (Varior-Krishnan, *et al.*, 1994). The envelope glycoprotein haemagglutinin (H) of MV is the ligand of CD46 (Gerlier, *et al.*, 1994). This interaction leads to a specific downregulation of CD46 by internalization. The H-mediated CD46 downregulation is reversible and restricted to CD46 since expression of other four surface markers (CD3, CD14, CD47 and CD63) is unaffected (Krantic, *et al.*, 1995). After binding to its target cell, MV has to fuse with the plasma membrane. Although virus envelope fusion to the cell membrane is difficult to detect, the MV-induced cell-to-cell fusion which is mediated by MV H and F glycoproteins and CD46 is likely to involve similar molecular mechanisms (Naniche, *et al.*, 1993).

Ligation of the CD46 receptor on PMNL by the CD46 antibody resulted in a decrease in the chemotaxis of PMNL. This decrease occurred in a dose-dependent manner; the highest reduction occurring in PMNL exposed to a higher dose of CD46 antibody. Also, apoptosis was found to be elevated in PMNL exposed to both the low and high concentration of this antibody. It is clear from these observations that occupation of the MV receptor, CD46, by a specific antibody, had a similar effect to that observed for the attachment of different MV strains to the receptor. Therefore, the mechanism(s) involved in the suppression of PMNL functions and induction of apoptosis appear to involve the binding of MV to its cellular receptor on PMNL. The significance of CD46 downregulation on PMNL following MV infection could be that the PMNL would become vulnerable to complement lysis, as the CD46 receptor would no longer be able to fulfill its main function of protecting the cell from complement-mediated destruction. The end result would be a reduction in both the number of circulating PMNL and in their functional capacity.

Taken together, these results show that MV has the ability to cause immune suppression in the host by directly incapacitating a number of PMNL functions and possibly reducing their numbers in the circulation through accelerated cell death (apoptosis). This most likely occurs at the stage of virus attachment. It is therefore likely that MV by binding to PMNL *in vivo* (in particular, the wild-type strain) plays an important role in compromising innate cellular immunity in the MV-

infected host and so predispose the individual to secondary microbial infections.

An understanding of the mechanisms involved in MV-induced suppression of PMNL function, particularly at the stage of virus binding could lead to means of protecting PMNL from the direct effects of MV, and so protect the host from secondary bacterial or fungal infections. Further studies are required to elucidate the precise mechanism by which MV incapacitates PMNL, and to determine to what extent attenuated MV vaccines may impact on an already compromised innate cellular (PMNL) immune response particularly in those children born to HIV-infected mothers that become infected within the first year of life.

REFERENCES

Aaby, P., Clements, J. and Orinda, V. (1991). *Mortality from measles : measuring the impact.* Geneva : Expanded Programme on Immunizations, World Health Organization.

Abramson, J.S. and Mills, E.L. (1988). *Depression of neutrophil function induced by viruses and its role in secondary microbial infection.* Rev. Infect. Dis., 10 (2), March-April; pp. 326-342.

Abramson, J.S. and Wheeler, J.G. (1994). *Virus-induced neutrophil dysfunction : role in the pathogenesis of bacterial infections.* Pediatr. Infect. Dis. J., 13 : 643-652.

Abramson, J.S., Giebink, G.S., Mills, E.L. and Quie, P.G. (1981). *Polymorphonuclear leukocyte dysfunction during Influenza virus infection in chinchillas.* J. Infect. Dis., 143 : 836-845.

Abramson, J.S., Giebink, G.S. and Quie, P.G. (1982). *Influenza A virus-induced polymorphonuclear leukocyte dysfunction in the pathogenesis of experimental pneumococcal otitis media.* Infect. Immun., 36 : 289-296.

Al-Attar, I., Reisman, J., Muehlmann, M. and McIntosh, K. (1995). *Decline of measles antibody titres after immunization in human immunodeficiency virus-infected children.* Pediatr. Infect. Dis. J., 14 : 149-150.

Albrecht, P., Ennis, F.A., Saltzman, E.J. and Krugman, S. (1977). *Persistence of maternal antibody in infants beyond 12 months : mechanism of measles vaccine failure.* J. Pediatr., 91 : 715-718.

Albrecht, P., Lorenz, D., Klutch, M.J., Vickers, J.H. and Ennis, F.A. (1980). *Fatal measles infection in marmosets : pathogenesis and prophylaxis.* Infect. Immun., 27 : 969-978.

Alkhatib, G. and Briedis, D.J. (1986). *The predicted primary structure of measles virus hemagglutinin.* Virology, 150 : 479-490.

Alkhatib, G., Shen, S-H., Briedis, D. et al. (1994). *Functional analysis of N-linked glycosylation mutants of the measles virus fusion protein synthesized by recombinant vaccinia virus vectors.* J. Virol., 68 : 1522-1531.

Anderson, R., Rabson, A.R., Sher, R. and Koornhof, H.J. (1976). *Defective neutrophil motility in children with measles.* J. Pediatr., 89 (1) : 27-32.

Arneborn, P. and Biberfeld, G. (1983). *T lymphocyte subpopulations in relation to immunosuppression in measles and varicella.* Infect. Immun., 39 : 29-37.

Arpadi, S.M., Markowitz, L.E., Baughman, A.L., et al. (1996). *Measles antibody in vaccinated human immunodeficiency virus type 1-infected children.* Pediatrics, 97 : 653-657.

Arya, L.S., Tanna, I., Tahiri, C., Saidali, A. and Singh, M. (1987). *Spectrum of complications of measles in Afghanistan : a study of 784 cases.* J. Trop. Med. Hyg., 90 : 117-122.

Baczko, K., Billeter, M. and ter Meulen, V. (1983). *Purification and molecular weight determination of measles virus genomic RNA.* J. Gen. Virol., 64 : 1409-1413.

Bale, J.F. Jr., Kern, E.R., Overall, J.C. Jr. and Glasgow, L.A. (1982). *Enhanced susceptibility of mice infected with murine cytomegalovirus to intranasal challenge with Escherichia coli : pathogenesis and altered inflammatory response.* J. Infect. Dis., 145 : 525-531.

Barclay, A.J.G., Foster, A. and Sommer, A. (1987). *Vitamin A supplements and mortality related to measles : a randomised clinical trial.* Br. Med. J., 294 : 294-296.

Beckford, A.P, Kaschula, R.O.C. and Stephen, C. (1985). *Factors associated with fatal cases of measles : a retrospective autopsy study.* S. Afr. Med. J., 68 : 858-863.

Bedows, E., Rao, K.M.K. and Welsh, M.J. (1983). *Fate of microfilaments in vero cells infected with measles virus and herpes simplex virus type 1.* Mol. Cell. Biol., 4 : 712-719.

Bellanti, J.A., Sanga, R.L, Klutinis, B. et al. (1969). *Antibody responses in serum and nasal secretions of children immunized with inactivated and attenuated measles virus vaccines.* N. Engl. J. Med., 280 : 628-633.

Bellini, W.J., Englund, G., Rozenblatt, S., Arnheiter, H. and Richardson, C.D. (1985). *Measles virus P gene codes for two proteins.* J. Virol., 53 : 908-919.

Bellini, W.J., McFarlin, D.E., Silver, G.D., Mingioli, E.S. and McFarland, H.F. (1981). *Immune reactivity of purified hemagglutinin of measles virus.* Infect. Immun., 32 : 1051-1057.

Bellini, W.J., Rota, J.S. and Rota, P.A. (1994). *Virology of measles virus.* Journal of infectious diseases, 170 (suppl. 1) : S15-S23.

Bellini, W.J., Silver, G.D. and McFarlin, D.E. (1983). *Biosynthesis of measles virus hemagglutinin in persistently infected cells.* Arch. Virol.,
75 : 87-101.

Benjamin, B. and Ward, S.M. (1932). *Leukocytic response to measles.* Am. J. Dis. Child.,
44 : 921-963.

Besser, G.M., Davis, J., Duncan, C., Kirk, B. and Kuper, S.W.A. (1967). *Glandular fever and specific viral infections : uptake of tritiated thymidine by circulating leucocytes.* Br. J. Haematol.,
13 : 189-193.

Birrer, M.J., Udem, S., Nathenson, S. and Bloom, B.R. (1981). *Antigenic variants of measles virus.* Nature, 293 : 67-69.

Black, F.L. (1975). *Infectious diseases in primitive societies.* Science, 187 : 515-518.

Black, F.L. (1989). *Measles active and passive immunity in a worldwide perspective.* Prog. Med. Virol., 36 : 1-33.

Black, F.L. and Rosen, L. (1962). *Patterns of measles antibodies in residents of Tahiti and their stability in the absence of re-exposure.* J. Immunol., 88 : 725-731.

Black, F.L. and Sheridan, S.E. (1960). *Studies on attenuated measles virus vaccine : IV. Administration of vaccine by several routes.* N. Engl. J. Med., 263 : 165-169.

Blumberg, B.M., Crowley, J.C., Silverman, J.J., Menonna, J., Cook, S.D. and Dowling, P.C. (1988). *Measles virus L protein evidences elements of ancestral RNA polymerase.* Virology, 164 : 487-497.

Bohn, W., Rutter, G., Hohenberg, H., Mannweiler, K. and Nobis, P. (1986) *Involvement of actin filaments in budding of measles virus : studies of cytoskeletons of infected cells.* Virology, 149 : 91-106.

Boxer, L.A. and Todd III, R.F. (1993). *Therapeutic modulation of neutrophil number and function.* In : The Neutrophil. Abramson, J.S. and Wheeler, J.G. (eds.). pp. 263-302.

Brodsky, A.L. (1972). *Atypical measles : severe illness in recipients of killed measles virus vaccine upon exposure to natural infection.* JAMA, 222 : 1415-1416.

Brown, D.W., Ramsay, M.E., Richards, A.F. and Miller, E. (1994). *Salivary diagnosis of measles : a study of notified cases in the United Kingdom, 1991-3.* Br. Med. J., 308 : 1015-1017.

Burnet, F.M. (1968). *Measles as an index of immunological function.* Lancet, 2 : 610-613.

Buynak, E.B., Peck, H.M., Creamer, A.A., Goldner, H. and Hilleman, M.R. (1962). *Differentiation of virulent from avirulent measles strains.* Am. J. Dis. Child. 103 : 291-303.

Carter, M.J. and ter Meulen, V. (1995). *Measles.* In : Principles and Practice of Clinical Virology (3rd edition). Zuckerman, A.J., Banatvala, J.E. and Pattison, J.R. (eds.). John Wiley and Sons, Ltd. pp. 337-361.

Carter, M.J., Willcocks, M.M., Loffler, S. and ter Meulen, V. (1982). *Relationships between monoclonal antibody-binding sites on the measles virus haemagglutinin.* J. Gen. Virol., 63 : 113-120.

Case Records of the Massachusetts General Hospital, (1974). N. Engl. J. Med., 291 : 141-149.

Castenada, S.J. and Wong, T.C. (1990). *Leader sequence distinguishes between translatable and encapsidated measles virus RNAs.* J. Virol., 64 : 222-230.

Cattaneo, R. and Rose, J.K. (1993). *Cell fusion by the envelope glycoproteins of persistent measles viruses which caused lethal human brain disease.* J. Virol., 67 : 1493-1503.

Centers for Disease Control, (1983). *Classification of measles cases and categorization of measles elimination programs.* MMWR, 31 : 707-711.

Centers for Disease Control, (1988). *Measles in HIV-infected children.* MMWR, 37 : 183-186.

Chen, T.P., Roberts, R.L., Wu, K.G., Ank, B.J. and Stiehm, E.R. (1993). *Decreased superoxide anion and hydrogen peroxide production by neutrophils and monocytes in human immunodeficiency virus-infected children and adults.* Pediatr. Res. 34 : 544-550.

Choppin, P.W., Richardson, C.D. and Merz, D.C. (1981). *The functions and inhibition of membrane glycoproteins of paramyxoviruses and myxoviruses, and the role of the measles virus M protein in subacute sclerosing panencephalitis.* J. Infect. Dis., 143 : 352-363.

Choppin, P.W. and Scheid, A. (1980). *The role of viral glycoproteins in adsorption, penetration and pathogenicity of viruses.* Rev. Infect. Dis., 2 : 40-61.

Christensen, P.E., Schmidt, H., Bang, H.O. et al. (1952). *Measles in virgin soil : II. The epidemic proper.* Acta Med. Scand., 144 : 430-449.

Clem, R.J. and Duckett, C.S. (1997). *The iap genes : unique arbitrators of cell death.* Trends in Cell Biol., 7 : 337-339.

Cohen, N.A. (1963). *Myocarditis in prodromal measles.* Am. J. Clin. Pathol., 40 : 50-53.

Collier, L. and Oxford, J. (1993). *Infections caused by Paramyxoviruses.* In : Human virology : A text for students of Medicine, Dentistry and Microbiology. pp. 111-121.

Condorelli, F. and Ziegler, T. (1993). *Dot immunobinding assay for simultaneous detection of specific immunoglobulin G antibodies to measles virus, mumps virus, and rubella virus.* J. Clin. Microbiol., 31 : 717-719.

Coovadia, H.M., Parent, M.A., Loening, W.E.K. et al. (1974). *An evaluation of factors associated with the depression of immunity in malnutrition and in measles.* Am. J. Clin. Nutr., 27 : 665-669.

Coutsoudis, A., Broughton, M. and Coovadia, H.M. (1991). *Vitamin A supplementation reduces measles morbidity in young African children : a randomized, placebo-controlled, double-blind trial.* Am. J. Clin. Nutr., 54 : 890-895.

Coutsoudis, A., Kiepiela, P., Coovadia, H.M. and Broughton, M. (1992). *Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles.* Pediatr. Infect. Dis. J., 11 : 203-209.

Crespi, M., Struthers, J.K., Smith, A.N., et al. (1988). *Interferon status after measles virus infection.* S. Afr. Med. J., 73 : 711-712.

Crowley, J.C., Dowling, P.C., Menonna, J., et al. (1988). *Sequence variability and function of measles virus 3´and 5´ends and intercistronic regions.* Virology, 164 : 498-506.

Cutts, F.T., Othepa, O., Vernon, A.A., et al. (1994). *Measles control in Kinshasa, Zaire improved with high coverage and use of medium titre EZ vaccine at age 6 months.* Int. J. Epidemiol., 23 : 624-631.

Degan, J.A. (1937). *Visceral pathology in measles.* Am. J. Med. Sci., 194 : 104-111.

De Jong, J.G. and Winkler, K.C. (1964). *Survival of measles virus in air.* Nature, 201 : 1054-1055.

Dhib-Jalbut, S.S. and Cowan, E.P. (1993). *Direct evidence that interferon-beta mediates enhanced HLA-class I expression in measles virus-infected cells.* J. Immunol., 151 : 1-11.

Diaz, T., Nunez, J.C., Rullan, J.V., Markowitz, L.E., Barker, N.D. and Horan, J. (1992). *Risk factors associated with severe measles in Puerto Rico.* Pediatr. Infect. Dis. J., 11 : 836-840.

Dorig, R.E., Marcil, A., Chopra, A. and Richardson, C.D. (1993). *The human CD46 molecule is a receptor for measles virus (Edmonston strain).* Cell, 75 : 295-305.

Dosseter, J., Whittle, H.C. and Greenwood, B.M. (1977). *Persistent measles infection in malnourished children.* British Medical Journal, 1 : 1633-1635.

Dowling, P.C., Blumber, B.J., Menonna, J., et al., (1986). *Transcriptional map of measles virus genome.* J. Gen. Virol., 67 : 1987-1992.

Dyer, I. (1940). *Measles complicating pregnancy.* S. Med. J., 33 : 601-604.

Ellis, M., Gupta, S., Galant, S., Hakim, S., VandeVen, C., Toy, C. and Cairo, M.S. (1988). *Impaired neutrophil function in patients with AIDS or AIDS-related complex : a comprehensive evaluation.* J. Infect. Dis., 158 : 1268-1276.

Embree, J.E., Datta, P., Stackiw, W., et al. (1992). *Increased risk of early measles in infants of human immunodeficiency virus type 1-seropositive mothers.* J. Infect. Dis., 165 : 262-267.

Enders, J.F., Katz, S.L. and Holloway, A. (1962). *Development of attenuated measles virus vaccines.* Am. J. Dis. Child., 103 : 335-340.

Enders, J.F., Katz, S.L., Milovanovic, M.V. and Holloway, A. (1960). *Studies of an attenuated measles virus vaccine : I. Development and preparation of the vaccine : technics for assay of effects of vaccination.* N. Engl. J. Med., 263 : 153-159.

Enders, J.F. and Peebles, T.C. (1954). *Propagation in tissue cultures of cytopathic agents from patients with measles.* Proc. Soc. Exp. Biol. Med., 86 : 277-286.

Enders, J.F., Peebles, T.C., McCarthy, K., Milovanovic, M., Mitus, A. and Holloway, A. (1957). *Measles virus : a summary of experiments concerned with isolation, properties, and behaviour.* Am. J. Public Health, 47 : 275-282.

Esolen, L.M., Ward, B.J., Moench, T.R. and Griffin, D.E. (1993). *Infection of monocytes during measles.* J. Infect. Dis., 168 : 47-52.

Finkle, H.E. (1964). *Measles myocarditis.* Am. Heart J., 67 : 679-683.

Font, R.L., Jenis, E.H. and Tuck, K.D. (1973). *Measles maculopathy associated with subacute sclerosing panencephalitis.* Arch. Pathol., 96 : 168-174.

Forni, A.L., Schluger, N.W. and Roberts, R.B. (1994). *Severe measles pneumonitis in adults : evaluation of clinical characteristics and therapy with intravenous ribavirin.* Clin. Infect. Dis., 19 : 454-462.

Fournier, J.G., Rozenblatt, S. and Bouteille, M. (1983). *Localization of measles virus nucleic acid sequences in infected cells by in situ hybridization.* Biol. Cell. 49 : 287-290.

Fragraeus, A., Örvell, C., Norberg, R. and Norrby, E. (1983). *Monoclonal antibodies to epitopes shared by actin and vimentin obtained by paramyxovirus immunization.* Exp. Cell. Res., 146 : 425-432.

Freeman, J.M. (1969). *The clinical spectrum and early diagnosis of Dawson's encephalitis.* J. Pediatr., 75 : 590-603.

Fujinami, R.S. and Oldstone, M.B.A. (1979). *Antiviral antibody reating on the plasma membrane alters measles virus expression inside the cell.* Nature, 279 : 529-530.

Fujinami, R.S. and Oldstone, M.B.A. (1980). *Alterations in expression of measles virus polypeptides by antibody : molecular events in antibody induced antigenic modulation.* J. Immunol., 125 : 78-85.

Fulginiti, V.A. and Kempe, C.H. (1963). *Measles exposure among vaccine recipients.* Am. J. Dis. C., 106 : 450-461.

Fulginiti, V.A., Arthur, J.H., Pearlman, D.S., et al. (1967). *Altered reactivity to measles virus.* Am. J. Dis. Child., 115 : 671-676.

Furukawa, S., Matsubara, T., Yone, K., Hirano, Y., Okumura, K. and Yabuta, K. (1992). *Kawasaki disease differs from anaphylactoid purpura and measles with regard to tumour necrosis factor-alpha and interleukin-6 in serum.* Eur. J. Pediatr., 151 : 44-47.

Gallagher, M.E., Welliver, R., Yamanaka, T. et al. (1981). *Cell-mediated immune responsiveness to measles.* Am. J. Dis. Child., 135 : 48-51.

Gazela, E., Karplus, M., Liberman, J.R., et al. (1985). *The effect of maternal measles on the fetus.* Pediatr. Infect. Dis., 4 : 203-204.

Gellini, B.G. and Katz, S.L. (1994a). *Measles : State of the art and future directions.* Journal of infectious diseases, 170 (suppl.1) : S3-S14.

Gellini, B.G. and Katz, S.L. (1994b). *Putting a stop to a serial killer : Measles.* J. Infect. Dis., 170 (suppl. 1) : S1-S2.

Gerald, C., Buckland, R., Barker, R., Freeman, G. and Wild, T.F. (1986). *Measles virus haemagglutinin gene : cloning, complete nucleotide sequence analysis and expression in cos cells.* J. Gen. Virol., 67 : 2695-2703.

Gerlier, D., Trescol-Biemont, M.C., Varior-Krishnan, G., Naniche, D., Fugier-Vivier, I. And Rabourdin-Combe, C. (1994). *Efficient MHC class II-restricted presentation of measles virus relies on haemagglutin-mediated targeting to its cellular receptorhuman CD46 expressed by murine B cells.* J. Exp. Med., 179 : 353-358.

Giraudon, P. and Wild, T.F. (1981). *Monoclonal antibodies against measles virus.* J. Gen. Virol., 54 : 325-332.

Giraudon, P, Jacquier, M.F. and Wild, T.F. (1988). *Antigenic analysis of African measles virus field isolates : identification and location of one conserved and two variable epitope sites on the NP protein.* Virus Res., 18 : 137-152.

Giraudon, P. and Wild, T.F. (1985). *Correlation between epitopes on hemagglutinin of measles virus and biological activities : passive protection by monoclonal antibodies is related to their hemagglutinin inhibiting activity.* Virology, 144 : 46-58.

Giustra, F.X. (1954). *Final report on a case of myocarditis following measles.* Am. J. Dis. Child., 87 : 615-618

Gombart, A.F., Hirano, A. and Wong, T.C. (1993). *Conformational maturation of measles virus nucleocapsid protein.* J. Virol., 67 : 4133-4141.

Good, R.A. and Zaki, S.J. (1956). *Disturbances in gamma globulin synthesis as experiments in nature.* Pediatrics, 18 : 109-149.

Gordon, J.E. and Ingalls, T.H. (1954). *Modern measles.* Am. J. Med. Sci., 228 : 334-361.

Graves, M.C. (1981). *Measles virus polypeptides in infected cells studied by immune precipitation and one-dimensional peptide mapping.* J. Virol., 38 : 224-230.

Graves, M., Griffin, D.E., Johnson, R.T. et al. (1984). *Development of antibody to measles virus polypeptides during complicated and uncomplicated measles virus infections.* J. Virol., 49 : 409-412.

Graziano, K.D., Ruckdeschel, J.C. and Mardinery, M.R. (1975). *Cell-associated immunity to measles (rubeola).* Cell. Immunol., 15 : 347-359.

Greenberg, B.L., Sack, R.B., Salazar-Lindo, L.E., et al. (1991). *Measles-associated diarrhoea in hospitalized children in Lima, Peru : pathogenic agents and impact on growth.* J. Infect. Dis., 163 : 495-502.

Gremillion, D.H. and Crawford, G.E. (1981). *Measles pneumonia in young adults : an analysis of 106 cases.* Am. J. Med., 71 : 539-542.

Griffin, D.E. and Bellini, W.J. (1996). ***Measles virus.*** In : Fields Virology (3rd edition). Fields, B.N., Knipe, D.M., Howley, P.M., et al. (eds.). Lippincott-Raven publishers, Philadelphia. pp. 1267-1312.

Griffin, D.E. and Ward, B.J. (1993). ***Differential CD4 T cell activation in measles.*** J. Infect. Dis., 168 : 275-281.

Griffin, D.E., Cooper, S.J., Hirsch, R.L. et al. (1985). ***Changes in plasma IgE levels during complicated and uncomplicated measles virus infections.*** J. Allergy Clin. Immunol., 76 : 206-213.

Griffin, D.E., Moench, T.R., Johnson, R.T., Lindo de Soriano, I. and Vaisberg, A. (1986). *Peripheral blood mononuclear cells during natural measles virus infection : cell surface phenotypes and evidence for activation.* Clin. Immunol. Immunopathol., 40 : 305-312.

Griffin, D.E., Ward, B.J. and Esolen, L.M. (1994). *Pathogenesis of measles virus infection : an hypothesis for altered immune responses.* The J. Infect. Dis., 170 (suppl. 1) : S24-S31.

Griffin, D.E., Ward, B.J., Jaurequi, E., Johnson, R.T. and Vaisberg, A. (1989). *Immune activation during measles.* N. Engl. J. Med. 320 : 1667-1672.

Griffin, D.E., Ward, B.J., Jaurequi, E., Johnson, R.T. and Vaisberg, A. (1990). *Immune activation during measles : interferon-gamma and neopterin in plasma and cerebrospinal fluid in complicated and uncomplicated disease.* J. Infect. Dis., 161 : 449-453.

Griffin, D.E., Ward, B.J., Jaurequi, E., Johnson, R.T. and Vaisberg, A. (1992). *Immune activation during measles : beta-2-microglobulin in plasma and cerebrospinal fluid in complicated and uncomplicated disease.* J. Infect. Dis., 166 : 1170-1173.

Hall, W.J. and Hall, C.B. (1979). *Atypical measles in adolescents : evaluation of clinical and pulmonary function.* Ann. Intern. Med., 90 : 882-886.

Hektoen, L. (1905). *Experimental measles.* J. Infect. Dis., 2 : 238-255.

Hirano, A., Wang, A.H., Gombart, A.F. and Wong, T.C. (1992). *The matrix proteins of neurovirulent subacute sclerosing panencephalitis virus and its acute measles virus progenitor are functionally different.* Proc. Natl. Acad. Sci. USA, 89 : 8745-8749.

Hirsch, R.L., Griffin, D.E., Johnson, R.T., et al. (1984). *Cellular immune responses during complicated and uncomplicated measles virus infections of man.* Clin. Immunol. Immunopathol., 31 : 1-12.

Home, F. (1759). *Medical facts and experiments.* London : H. Miller.

Hope-Simpson, R.E. (1952). *Infectiousness of communicable disease in the household (measles, chicken pox and mumps).* Lancet, 2 : 549-552.

Hosoya, M., Shigeta, S., Nakamura, K. and DeClercq, E. (1989). *Inhibitory effect of selected antiviral compounds on measles (SSPE) virus replication in vitro.* Antiviral Res., 12 : 87-97.

Hoyne, A.L. and Slotkowski, E.L. (1947). *Frequency of encephalitis as a complication of measles.* Am. J. Dis. Child., 73 : 554-558.

Huber, M., Cattaneo, R., Spielhofer, P., et al. (1991). *Measles virus phosphoprotein retains the nucleocapsid protein in the cytoplasm.* Virology, 185 : 299-308.

Jabbour, J.T., Duenas, D.A., Sever, J.L., et al. (1972). *Epidemiology of subacute sclerosing panencephalitis.* JAMA, 220 : 959-962.

Jabbour, J.T., Garcia, J.H., Lemmi, H., et al. (1969). *Subacute sclerosing panencephalitis : A multidisciplinary study of eight cases.* JAMA, 207 : 2248-2254.

Jacobson, M.A., Liu, R.C., Davis, D. and Cohen, P.E. (1997). *Human immunodeficiency virus disease-related neutropenia and the risk of hospitalization for bacterial infection.* Arch. Intern. Med., 157 : 1825-1831.

Jacobson, S. and McFarland, H.F. (1982). *Measles virus persistence in human lymphocytes : a role for virus induced interferon.* J. Gen. Virol., 63 : 351-357.

Jespersen, C.S., Littauer, J. and Sagild, U. (1977). *Measles as a cause of fetal defects : A retrospective study of ten measles epidemics in Greenland.* Acta Pediatr. Scand., 66 : 367-372.

Joffe, M.I. and Rabson, A.R. (1981). *Defective helper factor (LMC) production in patients with acute measles infection.* Clin. Immunol. Immunopathol., 20 : 215-223.

Johnson, K.P., Norrby, E., Swoveland, P., et al. (1984). *Experimental subacute sclerosing panencephalitis : selective disappearance of measles virus matrix protein from the central nervous system.* J. infect. Dis., 144 : 161-169.

Kalter, S.S., Herberling, R.L. and Barry, J.D. (1991). *Detection and titration of measles virus antibody by hemagglutination inhibiting and by dot immunobinding.* J. Clin. Microbiol., 29 : 202-204.

Kamahora, J. and Nii, S. (1961). *Pathological and immunological studies of monkeys infected with measles virus.* Arch. Ges. Virusforsch., 16 : 161-167.

Karnovsky, M.L. (1968). *The metabolism of leukocytes.* Semin. Hematol., 5 : 156.

Karzon, D.T., Rush, D. and Winkelstein, W. (1965). *Immunization with inactivated measles virus vaccine : effect of booster dose and response to natural challenge.* Pediatrics, 36 : 40-50.

Katz, S.L., Enders, J.F. and Holloway, A. (1960). *Studies on attenuated measles virus vaccine II. Clinical, virologic and immunologic effects on vaccine in institutionalized children.* N. Engl. J. Med., 263 : 159-161.

Katz, S.L., Enders, J.F. and Holloway, A. (1962). *Edmonston attenuated measles strain.* Am. J. Dis. Child., 103 : 340-344.

Katz, S.L., Enders, J.F. and Kibrick, S. (1959). *Immunization of children with a live attenuated measles virus.* Am. J. Dis. Child., 98 : 605-607.

Katz, S.L., Kempe, C.H., Black, F.L., et al. (1960). *Studies on an attenuated measles virus vaccine VIII. General summary and evaluation of the results of vaccination.* N. Engl. J. Med., 263 : 180-184.

Katz, S.L, Milovanovic, M.V. and Enders, J.F. (1958). *Propagation of measles virus in cultures of chick embryo cells.* Proc. Soc. Exp. Biol. Med., 97 : 23.

Kempe, C.H. and Fulginiti, V.A. (1965). *The pathogenesis of measles virus infection.* Arch. Ges. Virusforsch., 16 : 103-128.

Kempe, C.H., Ott, E.W., St. Vincent, L., et al. (1960). *Studies on an attenuated measles virus vaccine III. Clinical and antigenic effects of vaccine in institutionalized children.* N. Engl. J. Med., 263 : 162-165.

Kingsbury, D.W. (1974). *The molecular virology of paramyxoviruses.* Med. Microbiol. Immunol., 160 : 73-83.

Kohn, J.L. (1933). *Measles in newborn infants (maternal infection).* J. Pediatr., 3 : 176-180.

Koplik, H. (1896). *The diagnosis of the invasion of measles from a study of the exanthema as it appears on the buccal mucous membrane.* Arch. Pediatr., 13 : 918-922.

Krah, D.L. (1989). *Characterization of octyl glucoside-solubilized cell membrane receptors for binding measles virus.* Virology, 172 : 386-390.

Krantic, S., Gimenez, C. and Rabourdin-Combe, C. (1995). *Cell-to-cell contact via measles virus haemagglutinin-CD46 interaction triggers CD46 down-regulation.* J. Gen. Virol., 76 : 2793-2800.

Kress, S., Schluederberg, A.E., Hornick, R.B. et al. (1961). *Studies with live attenuated measles virus vaccine : II. Clinical and immunologic response of children in an open community.* Am. J. Dis. Child., 101 : 701-707.

Kreth, H.W., ter Meulen, V., Eckert, G. (1979). *Demonstration of HLA restricted killer cells in patients with acute measles.* Med. Microbiol. Immunol., 165 : 203-214.

Krugman, S. (1963). *Medical progress : the clinical use of gamma globulin.* N. Engl. J. Med., 269 : 195-201.

Krugman, S., Giles, J.P., Friedman, H. et al. (1965). *Studies on immunity to measles.* J. Pediatrics, 66 : 471-488.

Krugman, S., Ward, R. and Katz, S.L. (1977). *Infectious Diseases of Children,*(ed. 6). St. Louis, Mosby.

La Boccetta, A.C. and Tornay, A.S. (1964). *Measles encephalitis : Report of 61 cases.* Am. J. Dis. Child., 107 : 247-255.

Labowskie, R.J., Edelman, R., Rustigian, R. et al. (1974). *Studies of cell-mediated immunity to measles virus by in vitro lymphocyte-mediated cyto-toxicity.* J. Infect. Dis., 129 : 233-239.

Lazzarin, A., Uberti-Foppa, C., Galli, M., Mantovani, A., Poli, G., Franzetti, F. and Novati, R. (1986). *Impairment of polymorphonuclear leukocyte function in patients with acquired immunodeficiency syndrome and with lymphadenopathy syndrome.* Clin. Exp. Immunol., 65 : 105-111.

Leopardi, R., Vainionpaa, R., Hurme, M., et al. (1992). *Measles virus infection enhances IL-1β but reduces tumor necrosis factor-alpha expression in human monocytes.* J. Immunol., 149 : 2397-2401.

Levine, A.J. (1997). *P53, the cellular gatekeeper for growth and division.* Cell, 88(3) : 323-331.

Lievens, A.W. and Burnell, P.A. (1986). *Specific immunoglobulin M enzyme-linked immunosorbent assay for confirming the diagnosis of measles.* J. Clin. Microbiol., 391-394.

Lin, C.Y. and Hsu, H.C. (1986). *Histopathological and immunological studies in spontaneous remission of nephrotic syndrome after intercurrent measles infection.* Nephron, 42 : 110-115.

Linneman, C.C., Heg, M.E., Rotte, T.C. et al. (1973). *Measles IgM response during reinfection of previously vaccinated children.* J. Pediatr., 82 : 798-801.

Linneman, C.C., Rotte, T.C., Schiff, G.M. et al. (1972). *Seroepidemiologic study of a measles epidemic in a highly immunized population.* Am. J. Epidemiol., 95 : 238-246.

Liszewski, M.K. and Atkinson, J.P. (1991). *Membrane cofactor protein (MCP or CD46) : newest member of the regulators of complement activation gene cluster.* Ann. Rev. of Immunol. 9 : 431-455.

Litvak, A.M., Sands, I.J. and Gibel, H. (1943). *Encephalitis complicating measles : report of fifty-six cases with follow-up studies in thirty-two.* Am. J. Dis. Child., 65 : 265-295.

Lorenz, D. and Albrecht, P. (1980). *Susceptibility of Tamarins (Saguinus) to measles virus.* Lab. Anim. Sci., 30 : 661-665.

Lucas, C.J., Biddison, W.E., Nelson, D.L. and Shaw, S. (1982). *Killing of measles virus-infected cells by human cytotoxic T cells.* Infect. Immun., 38 : 226-232.

Lucke, B. (1918). *Postmortem findings in measles-bronchopneumonia and other acute infections.* JAMA, 70 : 2006-2011.

Lund, G.A., Tyrrell, D.L.J., Bradley, R.D. and Scraba, D.G. (1984). *The molecular length of measles virus RNA and the structural organization of measles nucleocapsids.* J. Gen. Virol., 65 : 1535-1542.

Machamer, C.E., Hayes, E.C., Gollobin, S.D. and Westfall, L.K. (1980). *Antibodies against the measles matrix polypeptide after clinical infection and vaccination.* Infect. Immun., 27 : 817-825.

Makhene, M.K. and Diaz, P.S. (1993). *Clinical presentations and complications of suspected measles in hospitalized children.* Pediatr. Infect. Dis. J., 12 : 836-840.

Malvoisin, E. and Wild, T.F. (1990). *Contribution of measles virus fusion protein in protective immunity : anti-F monoclonal antibodies neutralize virus infectivity and protect mice against challenge.* J. Virol., 64 : 5160-5162.

Malvoisin, E. and Wild, T.F. (1993). *Measles virus glycoproteins : studies on the structure and interaction of the haemagglutinin and fusion proteins.* J. Gen. Virol., 74 : 2365-2372.

Markowitz, L.E. and Bernier, R.H. (1987). *Immunization of young infants with Edmonston-Zagreb measles vaccine.* Pediatr. Infect. Dis., 6 : 809-812.

Markowitz, L.E., Chandler, F.W., Roldan, E.O., et al. (1988). *Fatal maesles pneumonia without rash in a child with AIDS.* J. Infect. Dis., 158 : 480-483.

Martin, D.B., Weiner, L.B., Nieburg, P.I. and Blair, D.C. (1979). *Atypical measles in adolescent and young adults.* Ann. Intern. Med., 90 : 877-881.

Mawhinney, H, Allen IV, Beare, J.M., et al. (1971). *Dysgammaglobulinemia complicated by disseminated measles.* Br. Med. J., 2 : 380-381.

McChesney, M.B. and Oldstone, M.B. (1989). *Virus-induced immunosuppression : infections with measles virus and human immunodeficiency virus.* Adv. Immunol., 45 : 335-380.

McCrumb, F.R., Kress, S., Saunders, E., et al. (1961). *Studies with live attenuated measles vaccine I. Clinical and immunologic responses in institutionalized children.* Am. J. Dis. Child., 101 : 689-700.

McFarlin, D.E., Bellini, W.J., Mingioli, E.S., Behar, T.N. and Trudgett, A. (1980). *Monospecific antibody to the haemagglutinin of measles virus.* J. Gen. Virol., 48 : 425-429.

McKimm-Breschkin, J.L., Breschkin, A.M. and Rapp, F. (1982). *Characterization of the Halle SSPE measles virus isolate.* J. Gen. Virol., 59 : 57-64.

Measles encephalitis-United States, 1962-1979 (1981). MMWR, 30 : 362-364.

Meddows-Taylor, S., Martin, D.J. and Tiemessen, C.T. (1998). *Reduced expression of interleukin-8 receptors A and B on polymorphonuclear neutrophils from persons with human immunodeficiency virus type 1 disease and pulmonary tuberculosis.* J. Infect. Dis. 177 : 921-930.

Meddows-Taylor, S., Martin, D.J. and Tiemessen, C.T. (1999). *Dysregulated production of interleukin-8 in individuals infected with human immunodeficiency virus type 1 and Mycobacterium tuberculosis.* Infect. Immun., 67 : 1251-1260.

Miller, D.L. (1964). *Frequency of complications of measles, 1963.* Br. Med. J., 2 : 75-78.

Milovanovic, M.V., Enders, J.F. and Mitus, A. (1957). *Cultivation of measles virus in human amnion cells and in developing chick embryo.* Proc. Soc. Exp. Biol. Med., 95 : 120-127.

Mitus, A., Enders, J.F., Craig, J.M., Holloway, A. (1959). *Persistence of measles virus and depression of antibody formation in patients with giant-cell pneumonia after measles.* N. Engl. J. Med., 261 : 882-889.

Mitus, A., Holloway, A., Evans, E.A., et al. (1962). *Attenuated measles vaccine in children with acute leukemia.* Am. J. Dis. Child., 103 : 413-418.

Modlin, J.F. (1991). *Measles virus.* In : Textbook of Human virology (2nd edition). Belshe, R.B. (ed.).

Modlin, J.F., Halsey, N.A., Eddins, D.L., et al. (1979). *Epidemiology of subacute sclerosing panencephalitis.* J. Pediatr., 94 : 231-236.

Moench, T.R., Griffin, D.E., Obriecht, C.R., Vaisberg, A.J. and Johnson, R.T. (1988). *Acute measles in patients with and without neurological involvement : distribution of measles virus antigen and RNA.* J. Infect. Dis., 158 : 433-442.

Morgan, E.M. and Rapp, F. (1977). *Measles virus and its associated diseases.* Bacteriol. Rev., 41 : 636-666.

Morley, D. (1969). *Severe measles in the tropics.* Br. Med. J., 1 : 297-300.

Mowat, A.G. and Baum, J. (1971a). *Polymorphonuclear leukocyte chemotaxis in patients with bacterial infections.* Br. Med. J., 3 : 617.

Mowat, A.G. and Baum, J. (1971b). *Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus.* N. Engl. J. Med., 284 : 621.

Murphy, M.F. (1978). *In vitro inhibition of subacute sclerosing panencephalitis virus by the antiviral agent ribavirin.* J. Infect. Dis., 138 : 249-251.

Murphy, P.M., Lane, H.C., Fauci, A.S. and Gallin, J.I. (1988). *Impairment of neutrophil bactericidal capacity in patients with AIDS.* J. Infect. Dis., 158 : 627-630.

Murphy, J.V., Yunis, E.J. and Turner, M. (1974). *Rapidly progressive subacute sclerosing panencephalitis.* Presented to the Third Annual Meeting of the Child Neurology Society, Madison, Wisc., Oct. 10-12, 1974.

Myou, S., Fujimura, M., Yasui, M., Ueno, T. and Matsuda, T. (1993). *Bronchoalveolar lavage cell analysis in measles viral pneumonia.* Eur. Resp. J., 6 : 1437-1442.

Nader, P.R., Howitz, M.S. and Rousseau, J. (1968). *Atypical exanthem following exposure to natural measles : eleven cases in children previously inoculated with killed vaccine.* J. Pediatr., 72 : 22-28.

Nagata, S. (1997). *Apoptosis by death factor.* Cell, 88(3) : 355-365.

Nahmias, A.J., Griffin, D., Salsburg, C. et al. (1967). *Thymic aplasia with lymphopenia, plasma cells, and normal immunoglobulins.* JAMA, 201 : 729-734.

Naniche, D., Varior-Krishnan, G, Cervoni, F. et al. (1993). *Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus.* J. Virol., 67 : 6025-6032.

Naniche, D., Wild, T.F., Rabourdin-Combe, C. and Gerlier, D. (1992). *A monoclonal antibody recognizes a human cell surface glycoprotein involved in measles virus binding.* J. Gen. Virol., 73 : 2617-2624.

Norrby, E. (1962). *Hemagglutination by measles virus.* Arch. Ges. Virusforch., 12 : 164-172.

Norrby, E. and Gollmar, Y. (1972). *Appearance and persistence of antibodies against different virus components after regular measles infections.* Infect. Immun., 6 : 240-247.

Norrby, E. and Gollmar, Y. (1975). *Identification of measles virus-specific hemolysis-inhibiting antibodies separate from hemagglutination-inhibiting antibodies.* Infect. Immun., 11 : 231-239.

Norrby, E., Lagercrantz, R., Gard, S. and Carlstrom, G. (1965). *Measles vaccination : III. Serologic responses to immunization with purified hemagglutinin.* Acta. Paediatr. Scand., 54 : 581-586.

Norrby, E. and Oxman, M.N. (1990). *Measles virus.* In : Fields' Virology, vol. 1 : 1013-1044. Fields, B.N., Knipe, D.M., Chanock, R.M., et al. (eds.). New York : Raven Press.

Nur, Y.A., Groven, J., Yusuf, M.A. and Osterhaus, A.D.M.E. (1999). *IgM antibodies in hospitalized children with febrile illness during an inter-epidemic period of measles, in Somalia.* J. Clin. Virol., 12 : 21-25.

O'Donovan, C. (1971). *Measles in Kenyan children.* East Afr. Med. J., 48 : 526-532.

Oddo, F.G., Flaccomio, R. and Sinatra, A. (1961). *"Giant cell" and "strand-forming" cytopathic effect of measles virus lines conditioned by serial propagation with diluted or concentrated inocculum.* Virology, 13 : 550-553.

O'Neil, G.C., Stokes, J. Jr. and Shaffer, M.F. (1940). *Virus of measles grown in developing chick embryo.* Am. J. Dis. Child., 60 : 757-772.

Ono, K., Iwa, N., Kato, S. and Konobe, T. (1970). *Demonstration of viral antigen in giant cells formed in monkeys experimentally infected with measles virus.* Biken J., 13 : 329-337.

Ordman, C.W., Jennings, C.G. and Janeway, C.A. XII (1944). *The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles.* J. Clin. Invest., 23 : 541-549.

Örvell, C. (1994). *Measles virus.* In : Encyclopedia of Virology, vol. 2 (eds. Webster, R.G. and Granoff, A.). Academic Press.

Oshitani, H., Suzuki, H., Mpabalwani, M.E., Mizuta, K. and Numazaki, Y. (1996). *Measles case fatality by sex, vaccination status, and HIV-1 antibody in Zambian children.* Lancet, 348 : 415.

Oxman, M.N. (1997). In : Clinical Virology. Richman, D.D., Whitely, R.J. and Hayden, F.G. (eds.). Churchill Livingstone, New York.

Oxtoby, I.M., Ryder, R., Mvula, M., Nsa, W., Baende, E. and Onorato, I. (1989). *Patterns of immunity to measles among African children infected with the human immunodeficiency virus.* Epidemic Intelligence Service Conference Abstracts.

Panum, P.L. (1939). *Observations made during the epidemic of measles on Faroe Islands in the year 1846.* Med. Class 3 : 829-886.

Pather, M., Wesley, A.G., Schonland, M. et al. (1976). *Severe measles-associated pneumonia treated with assisted ventilation.* S. Afri. Med. J., 50 : 1600-1603.

Peakman, M. and Vergani, D. (1997). *Innate immunity II : cellular mechanisms.* In : Basic and Clinical Immunology. Peakman, M. and Vergani, D. (eds.). Churchill Livingstone, pp. 21-29.

Peries, J.R. and Chancy, C. (1962). *Studies on measles viral hemagglutination.* Proc. Soc. Exp. Biol. Med., 11 : 477-482.

Perry, K.R., Brown, D.W.G., Parry, J.V., Panday, S., Pipkin, C. and Richards, A. (1993). *Detection of measles, mumps and rubella antibodies in saliva using antibody capture radioimmunoassay.* J. Med. Virol., 40 : 235-240.

Petralli, J.K., Merigan, T.C. and Wilbur, J.R. (1965). *Circulating interferon after measles vaccination.* N. Engl. J. Med. 273 : 198-201.

Pette, M., Liebert, U.G., Gobel, U., Grosse-Wilde, H., Hartung, H-P. And Toyka, K.V. (1993). *Measles virus-directed responses of CD4+ T lymphocytes in MS patients and healthy individuals.* Neurology, 43 : 2019-2025.

Pitrak, D.L., Bak, P.M., De Marais, P., Novak, R.M. and Andresen, B.R. (1993). *Depressed neutrophil superoxide production in human immunodeficiency virus infection.* J. Infect. Dis., 167 : 1406-1410.

Portner, A. and Bussel, R.H. (1973). *Measles virus ribonucleic acid and protein synthesis : Effects of 6-azauridine and cycloheximide on virus replication.* J. Virol., 11 : 46-53.

Rammohan, K.W., McFarland, H.F. and McFarlin, D.E. (1982). *Subacute sclerosing panencephalitis after passive immunization and natural measles infection : role of antibody in persistence of measles virus.* Neurology, 32 : 390-394.

Rao, L. and White, E. (1997). *Bcl-2 and the ICE family of apoptotic regulators : making a connection.* Curr. Opin. Genet. Dev., 7(1) : 52-58.

Rauch, L.W. and Schmidt, R. (1965). *Measles immunization with killed virus vaccine.* Am. J. Dis. Child., 109 : 232-237.

Ray, J. and Fujinami, R.S. (1987). *Characterization of in vitro transcriptional products of measles virus.* J. Virol. 61 : 3381-3387.

Reissig, M., Black, F.L. and Melnick, J.L. (1956). *Formation of multinucleated giant cells in measles virus infected cultures deprived of glutamine.* Virology, 2 : 836-838.

Richardson, C.D., Berkovich, A., Rozenblatt, S. and Bellini, W.J. (1985). *Use of antibodies directed against synthetic peptides for identifying cDNA clones, establishing reading frames and deducing the gene order of measles virus.* J. Virol., 54 : 186-193.

Riley, E.C., Murphy, G. and Riley, R.L. (1978). *Airborne spread of measles in a suburban elementary school.* Am. J. Epidemiol., 107 : 421-432.

Rima, B., Earle, J.A.P., Baczko, K., Rota, P.A. and Bellini, W.J. (1995). *Measles virus strains variations.* Cur. Topics Microbiol. Immunol., 191.

Ritossa, P. and Mule, F. (1941). *Versuche zur Zuchtung des Masernvirus auf der chorionallantois des Huhnerembryos.* Arch. Ges. Virusforsch., 2 : 53-70.

Robb, R.M. and Walters, G.V. (1970). *Opthalmic manifestations of subacute sclerosing panencephalitis.* Arch. Ophthalmol., 83 : 426-435.

Robbins, S.J. and Bussel, R.H. (1979). *Structural phosphoproteins associated with purified measles virions and cytoplasmic nucleocapsids.* Intervirology, 12 : 96-102.

Rod, T., Haug, K.W. and Ulstrup, J.C. (1970). *Atypical measles after vaccination with killed vaccine.* J. Infect. Dis., 2 : 161-165.

Rose, J.W., Bellini, W.J., McFarlin, D.E. and McFarland, H.F. (1984). *Human cellular immune response to measles virus polypeptides.* J. Virol., 49 : 988-991.

Rossier, E., Miller, H., McCulloch, B., Sullivan, L. and Ward, K. (1991). *Comparison of immunofluorescence and enzyme immunoassay for detection of measles-specific immunoglobulin M antibody.* J. Clin. Microbiol., 29 : 1069-1071.

Rota, J.S., Wang, Z.D., Rota, P.A. and Bellini, W.J. (1994). *Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains.* Virus Res., 31 : 317-330.

Rozenblatt, S., Eizenberg, O., Ben-Levy, R., Lavie, V. and Bellini, W.J. (1985). *Sequence homology within the morbilliviruses.* J. Virol., 53 : 684-690.

Ruckdeschel, J.C., Graziano, K.D. and Mardiney, M.R. (1975). *Additional evidence that the cell-associated immune system is the primary host defence against measles.* Cell. Immunol., 17 : 11-18.

Ruckle, G. and Rogers, K.D. (1957). *Studies with measles virus II. Isolation of virus and immunologic studies in persons who have had the natural disease.* J. Immunol. 78 : 341-355.

Russel, S.M., Loveland, B.E., Johnstone, R.W., Thorley, B.R. and McKenzie, I.F.C. (1992). *Functional characterization of alternatively spliced CD46 cytoplasmic tails.* Transplantation Proceedings. 24 : 2329-2330.

Sabin, A.B. (1991). ***Measles, killer of millions in developing countries : strategy for rapid elimination and continuing control.*** Eur. J. Epidemiol., 7 : 1-22.

Sakata, H., Kobune, F., Sato, T.A., Tanabayahi, K., Yamada, A. and Sugiura, A. (1993). ***Variation in field isolates of measles virus during an 8-year period in Japan.*** Microbiol. Immunol., 37 : 233-237.

Savini, E. (1923). ***Essais de vaccinotherapie preventive dans le typhus exanthematique, la scarlatine et la rougeole.*** Compt. Rend. Soc. Biol., 89 : 694-696.

Scheifele, D.W. and Forbes, C.E. (1972). ***Prolonged giant cell excretion in severe African measles.*** Pediatrics, 50 : 867-873.

Schluederberg, A. (1965a). ***Immunoglobulins in viral infections.*** Nature, 205 : 1232-1233.

Schneider-Schaulies, S., Liebert, U.G., Baczko, K. and ter Meulen, V. (1990). ***Restricted expression of measles virus in primary rat astroglial cells.*** Virology, 177 : 802-806.

Schulz, T.F., Hoad, J.G., Whitby, D., Tizard, J., Dillon, M.J. and Weiss, R.A. (1992). *A measles virus isolate from a child with Kawasaki disease : sequence comparison with contemporaneous isolates from 'classical cases'.* J. Gen. Virol., 73 : 1581-1586.

Schwartz, A.J.F. (1962). *Preliminary tests of a highly attenuated measles vaccine.* Am. J. Dis. Child., 103 : 216-219.

Seligman, S.J. and Rapp, F. (1959). *A variant of measles virus in which giant cell formation appears to be genetically determined.* Virology, 9 : 143-145.

Shalekoff, S., Tiemessen, C.T., Gray, C.M. and Martin, D.J. (1998). *Depressed phagocytosis and oxidative burst in polymorphonuclear leukocytes from individuals with pulmonary tuberculosis with or without human immunodeficiency virus type 1 infection.* Clin. Diagn. Lab. Immunol., 5 : 41-44.

Sheshberadaran, H., Chen, S-H and Norrby, E. (1983). *Monoclonal antibodies against five structural components of measles virus.* Virology, 52 : 995-999.

Sheshberadaran, H. and Norrby, E. (1986). *Characterization of epitopes on the measles virus hemagglutinin.* Virology, 152 : 58-65.

Shigeta, S., Mori, S., Baba, M., et al. (1992). *Antiviral activities of ribavirin, 5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide, and 6'-(R)-6'-C-methylneplanocin A against several ortho- and paramyxoviruses.* Antimicrob. Agents Chemother., 36 : 435-439.

Shiozawa, S., Yoshikawa, N., Iijima, K. and Negishi, K. (1988). *A sensitive radioimmunoassay for circulating alpha-interferon in the plasma of healthy children and patients with measles virus infection.* Clin. Exp. Immunol., 73 : 366-369.

Sieber, G.R., Werner, B.G., Hasley, N.A. et al. (1993). *Interference of immune globulin with measles and rubella immunization.* J. Pediatr., 122 : 204-211.

Siegal, M.M., Walker, T.K. and Ablin, A.R. (1977). *Measles pneumonia in childhood leukemia.* Pediatrics, 60 : 38-40.

Siegel, M. (1973). *Congenital malformations following chickenpox, measles, mumps, and hepatitis.* JAMA, 226 : 1521.

Siegel, M. and Fuerst, H.T. (1966). *Low birth weight and maternal viral diseases : A prospective study of rubella, measles, mumps, chicken-pox, and hepatitis.* JAMA, 197 : 88.

Sissons, J.G.P., Colby, S.D., Harrison, W.O. and Oldstone, M.B.A. (1985). *Cytotoxic lymphocytes generated in vivo with acute measles virus infection.* Clin. Immunol. Immunopathol., 34 : 60-68.

Stallcup, K.C., Raine, C.S. and Fields, B.N. (1983). *Cytochalasin B inhibits the maturation of measles virus.* Virol., 124 : 59-74.

Stallcup, K.C., Wechsler, S.L. and Fields, B.N. (1979). *Purification of measles virus and characterization of subviral components.* J. Virol., 30 : 166-176.

Starr, S. and Berkovich, S. (1964). *Effects of measles, gamma-globulin-modified measles and vaccine measles on the tuberculin test.* N. Engl. J. Med., 270 : 386-391.

Suryanarayana, K., Baczko, K., ter Meulen, V., Wagner, R.R (1994). *Transcription inhibition and other properties of matrix proteins expressed by M genes cloned from measles viruses and diseased human brain tissue.* J. Virol., 68 : 1532-1543.

Takenhara, K., Hashimoto, H., Ri, T., Mori, T. and Yoshimura, M. (1992). *Characterization of baculovirus-expressed hemagglutinin and fusion glycoproteins of the attenuated measles virus strain A1K-C.* Virus Res., 26 : 167-175.

Tamashiro, V.G., Perez, H.H. and Griffin, D.E. (1987). *Prospective study of the magnitude and duration of changes in tuberculin reactivity during complicated and uncomplicated measles.* Pediatr. Infect. Dis. J., 6 : 451-454.

Tamin, A., Rota, P.A., Wang Z-D., Heath, J.L., Anderson, L.J. and Bellini, W.J. (1994). *Antigenic analysis of current wild type and vaccine strains of measles virus.* J. Infect. Dis., 170 : 795-801.

Tannous, R. and Meyers, M.G. (1979). *Acquired chemotactic inhibitors during infection with guinea pig cytomegalovirus and herpes simplex virus infection.* N. Engl. J. Med., 300 : 1345-1349.

Taylor, M.J., Godfrey, E., Baczko, K., ter Meulen, V., Wild, T.F. and Rima, B.K. (1991). *Identification of several different lineages of measles virus.* J. Gen. Virol., 72 : 83-88.

Teodoro, J.G. and Branton, P.E. (1997). *Regulation of apoptosis by viral gene products.* J. Virol., 71(3) : 1739-1746.

Thompson, C.B.(1995). *Apoptosis in the pathogenesis and treatment of disease.* Science.267 : 1456-1462.

Tyrrell, D.L. and Ehrnst, A. (1979). *Transmembrane communication in cells chronically infected with measles virus.* J. Cell. Biol., 81 : 396-402.

Tyrrell, D.L. and Norrby, E. (1978). *Structural polypeptides of measles virus.* J. Gen. Virol.,39: 219-229.

Udem, S.A. and Cook, K.A. (1984). *Isolation and characterization of measles virus intracellular nucleocapsid RNA.* J. Virol., 49 : 57-65.

Uylangco, C.V., Beroy, G.J., Santiago, L.T., Mercoleza, V.D. and Mendoza, S.L. (1981). *A double-blind, placebo-controlled evaluation of ribavirin in the treatment of acute measles.* Clin. Ther., 3 : 389-396.

Valone, F.H., Payan, D.G., Abrams, D.J. and Goetzl, E.J. (1984). *Defective polymorphonuclear leukocyte chemotaxis in homosexual men with persistent lymph node syndrome.* J. Infect. Dis., 150 : 267-271.

Van Binnendjik, R.S., Poelen, M.C.M., Kuijpers, K.C., Osterhaus, A.D.ME. and Uytdehaag, F.G.C.M. (1990). *The predominance of CD8+ T cells after infection with measles virus suggests a role for CD8+ class I MHC-restricted cytotoxic T lymphocytes (CTL) in recovery from measles.* J. Immunol., 144 : 2394-2399.

Van Binnendjik, R.S., Van der Heijden, R.W.J., Van Amerongen, G., Uytdehaag, F.G.C.M. and Osterhaus, A.D.M.E. (1994). *Viral replication and development of specific immunity in macaques after infection with different measles virus strain.* J. Infect. Dis., 170 : 443-448.

Van Binnendjik, R.S., Versteeg-van Oosten, J.P.M., Poelen, M.C.M. et al. (1993). *Human HLA class I- and HLA class II-restricted cloned cytotoxic T lymphocytes identify a cluster of epitopes on the measles virus fusion protein.* J. Virol., 67 : 2276-2284.

Varior-Krishnan, G., Trescol-Biemont, M-C, Naniche, D., Rabourdin-Combe, C. and Gerlier, D. (1994). *Glycosyl-phosphatidylinositol-anchored and transmembrane forms of CD46 display similar measles virus receptor properties : virus binding, fusion and replication, and down-regulation by haemagglutinin; and virus uptake and endocytosis for antigen presentation by Major Histocompatibility Complex class II molecules.* J. Virol. 68(12) : 7891-7899.

Varsanyi, T.M., Utter, G. and Norrby, E. (1984). *Purification, morphology and antigenic characterization of measles virus envelope components.* J. Gen. Virol., 65 : 355-366.

Vaux, D.L. and Strasser, A. (1996). *The molecular biology of apoptosis.* Proc. Natl. Acad. Sci. USA, 93(6) : 2239-2244.

Vialard, J., Lalumiere, M., Vernet, T. et al. (1990). *Synthesis of the membrane fusion and hemagglutinin proteins of measles virus, using a novel baculovirus vector containing the beta-galactosidase gene.* J. Virol., 64 : 37-50.

Volckaert-Vervliet, G. and Billiau, A. (1977). *Induction of interferon in human lymphoblastoid cells by Sendai and measles viruses.* J. Gen. Virol., 37 : 199-203.

Von Pirquet, C. (1908). *Verhalten der kutanen tuberkulin-reaktion wahrend der Masern.* Dtsch. Med. Wochenschr., 34 : 1297-1300.

Ward, B.J. and Griffin, D.E. (1993). *Changes in cytokine production after measles virus vaccination : predominant production of IL-4 suggests induction of a Th2 response.* Clin. Immunol. Immunopathol., 67 : 171-177.

Ward, B.J., Johnson, R.T., Vaisberg, A., Jaurequi, E. and Griffin, D.E. (1990). *Spontaneous proliferation of peripheral mononuclear cells in natural measles virus infection : identification of dividing cells and correlation with mitogen responsiveness.* Clin. Immunol. Immunopathol., 55 : 315-326.

Ward, B.J., Johnson, R.T., Vaisberg, A., et al. (1991). *Cytokine production in vitro and the lymphoproliferative defect of natural measles virus infection.* Clin. Immunol. Immunopathol., 61 : 236-248.

Warren, J. and Gallian, M.J. (1962). *Concentrated inactivated measles virus vaccine . preparation and antigenic potency.* Am. J. Dis. Child., 103 : 248-253.

Waters, D.J., Hersh, R.F. and Bussel, R.H. (1972). *Isolation and characterization of measles nucleocapsid from infected cells.* Virology, 48 : 278-281.

Waters, D.J., Hersh, R.F. and Bussel, R.H. (1972). *Isolation and characterization of measles nucleocapsid from infected cells.* Virology, 48 : 278-281.

Watson, G.I. (1965). *Serological studies on second attacks of measles and rubella.* Lancet, 1 : 80-81.

Welliver, R.C., Cherry, J.D. and Holtzman, A.E. (1977). *Typical, modified, and atypical measles. An emerging problem in the adolescent and adult.* Arch. Intern. Med., 137 : 39-41.

Wesley, A.G., Coovadia, H.M. and Henderson, L. (1978). *Immunological recovery after measles.* Clin. Exp. Immunol., 32 : 540-544.

Wesley, A.G., Coovadia, H.M. and Kiepiela, P. (1982). *Further predictive indices of clinical severity of measles.* S. Afri. Med. J., 61 : 663-665.

White, E. (1996). *Life, death and the pursuit of apoptosis.* Genes Dev., 10(1) : 1-15.

Whitley, R.J., Soong, S-J, Linneman, C. Jr. et al. (1982). *Herpes simplex : clinical assessment.* JAMA, 247 : 317-320.

Wild, T.F., Malvoisin, E. and Buckland, R. (1991). *Measles virus : both the haemagglutinin and fusion glycoproteins are required for fusion.* J. Gen. Virol., 72 : 439-442.

Winkelstein, W., Karzon, D.T., Rush, D., et al. (1965). *A field trial of inactivated measles virus vaccine in young school children.* JAMA, 194 : 494-498.

Wu, V.H., McFarland, H., Mayo, K., Hanger, L., Griffin, D.E. and Dhib-Jalbut, S. (1993). *Measles virus-specific cellular immunity in patients with vaccine failure.* J. Clin. Microbiol., 31 : 118-122.

Wyllie, A.H. (1980). *Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation.* Nature, 284(5756) : 555-556.

Young, L.W., Smith, D.I. and Glasgow, L.A. (1970). *Pneumonia of atypical measles. Residual nodular lesions.* A.J.R., 110 : 439-448.

Yourtee, E.L., Bia, F.J., Griffith, B.P. and Root, R.K. (1982). *Neutrophil response and function during acute cytomegalovirus infection in guinea pigs.* Infect. Immun., 36 : 11-16.

APPENDIX

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
C	C protein
CDC	Centre for Disease Control
CDV	Canine Distemper Virus
CMV	Cytomegalovirus
CPE	Cytopathic effects
CSF	Cerebrospinal Fluid
CYT	Cytoplasmic Tail
DHR	Dihydrorhodamine
DTH	Delayed type hypersensitivity
EBV	Epstein Barr Virus
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
F	Fusion protein
FCS	Foetal Calf Serum
h	hour
H	Haemagglutinin
HI	Heat Inactivated
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICE	Interleukin-1β Converting Enzyme
IF	Immunofluorescence
IFN	Interferon
Ig	Immunoglobulin
IL-8	Interleukin-8
ISG	Immune Serum Globulin

IVIG	Intravenous Immune Globulin
Kda	Kilodalton
L	Large protein
M	Matrix protein
mAb	monoclonal antibody
MHC	Major Histocompatibility Complex
MV	Measles Virus
N	Nucleocapsid
NK	Natural Killer cells
nm	nanometre
OD	Optical Density
P	Phosphoprotein
PBMCs	Pheripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PDV	Phocine Distemper Virus
PMNL	Polymorphonuclear leukocytes
R	Rhodamine
RBC	Red Blood Cells
ROI	Reactive Oxygen Intermediates
rpm	revolutions per minute
RT	Reverse Transcriptase
SABTS	South African Blood Transfusion Services
SCR	Short Consensus Repeat
SEM	Standard Error of the Mean
SSPE	Subacute Sclerosing Panencephalitis
STP	Serine, Threonine, Proline
TNF	Tumour Necrosis Factor
V	Variable protein
WHO	World Health Organisation