THE IMMUNO-HISTOCHEMICAL DIFFERENTIATION OF HUMAN LYMPHOID CELLS

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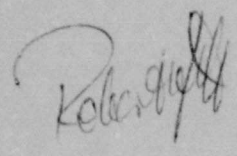
DECLARATION

I hereby declare that this dissertation is my own work and that it has not been submitted to any other university.



R.P.H. Hill

ERRATUM

Further discussions are necessary on some statements made in the text of this dissertation.

Practical applications of the fluorescein-conjugated anti-T cell reagent are illustrated by the way the remainder of the preparation was used subsequent to completion of this work.

With the cooperation of an interested pathologist the reagent was used in conjunction with EAC rosettes for B cells and EA rosettes for macrophages, to investigate three cases of lymphoid neoplasia. Cryostat sections of surgical material were examined by the three techniques in parallel. Two cases were thereby proved to be of B cell origin with EAC rosette distribution and T cell fluorescence being complementary in the disturbed architecture of the lymph nodes. The third case of splenic material gave an ambiguous distribution pattern with both T and B cells increased. A definite diagnosis was not made.

These elementary investigations illustrate how useful techniques for typing lymphocytes in tissue sections can be. Neither EAC rosette formation, nor this particular antiserum is capable of identifying individual cells, but more potent antisera produced by longer immunisation schedules as discussed in the text would overcome this problem as far as T cells are concerned.

The observation on p 77 line 15 that " Complement did not

appear necessary for anti thymocyte cytotoxicity", is a lesson in misinterpretation of the results of an ill-designed experiment. The function of complement in the antigen-antibody interaction is discussed on p 18 of the text and it is fundamental to immunology that the immunoglobulin molecule is not capable of cytotoxic function on it's own.

The error occurred during investigations into the cytotoxic activity of the anti-thymocyte antisera. These were set up in a standard technique (No. 14, p 56), with complement included in the system. A typical result is shown in figure 9, p 80, with a plateau of activity to a titre of 1:8 against viable thymocytes. These data indicated that the antisera were satisfactorily cytotoxic for their specific target cells, but unfortunately it was decided to investigate the role of complement in the system; an enquiry not of particular relevance to the overall object of work.

As discussed on pp 28 and 30 the experiences of other workers were encountered in the difficulty of obtaining and preserving viable thymocytes, particularly from human sources. Viable fetuses were rare, and frozen preparations lost viability much quicker than lymphatic leukaemia cells or peripheral blood lymphocytes. Thus, live thymocytes were used with far greater respect and economy.

Shortage of thymocytes was a restriction posed in setting up the secondary investigation on the role of complement. The three anti-T cell sera were only tested at a single dilution of 1:2 in duplicate, against viable thymocytes. Each serum was tested with complement and without complement in the system and controls were run to establish cytotoxicity of

complement alone; of inactivated normal rabbit serum, and any change in target cell viability during the test.

The results of this experiment were in accordance with the statement on p 77. However, if viable thymocytes had been more freely available, and if the investigation had had more importance, the antisera would have been tested over a range of dilutions to perhaps 1:64. With complement added, the results would have been similar to those illustrated in fig. 9. Without complement, the cytotoxic index would have fallen very quickly on dilution. The dependence of the anti-T cell antibodies on complement for full cytotoxic expression would have been quite obvious and the ambiguous statement would not have been made. To this effect the following lines should be deleted from the text.

p 77 line 15 (from "Complement) to line 22.

p 105 line 20 (from "Cytotoxicity) to line 24.

SUMMARY

The work describes the attempt to produce rabbit antisera capable of demonstrating human T and B cells in tissue sections by direct fluorescent antibody techniques. Thymus cells from therapeutically aborted fetuses, and lymphocytes from peripheral blood of patients suffering from chronic lymphatic leukaemia were characterised by conventional cell marker systems and stored as frozen living cells.

Antisera were raised in rabbits by intravenous inoculations of live cell suspensions. The resulting hyper-immune antibodies were recovered and purified as globulin solutions. These were examined for cytotoxicity for lymphocyte suspensions by trypan blue exclusion tests. Inhibition of function was studied by exposing normal lymphocytes to the antibodies and studying their capability to form sheep red cell rosettes.

The most suitable absorbents for removal of non-specific reactions were investigated using aliquots of globulin solutions. After bulk absorptions the antibodies were conjugated with fluorescein isothiocyanate. Column chromatography through Sephadex and DEAE cellulose beds was used to remove free fluorochrome and to recover optimally conjugated molecules. Fractions of effluent from the columns were subjected to ultraviolet light spectrophotometry and direct fluorescent antibody tests on normal human lymph node and thymus. Those fractions showing optimal physico-chemical data and biological performance were concentrated, pooled and freeze-dried for storage.

Antisera raised with thymus cells yielded a good reagent, cytotoxic for T cells, inhibiting sheep red cell rosette func-

tion and staining T cell membranes in thymus and T cell dependent areas of lymph node.

The anti-B cell reagent,however,was not successful. Antibody activity was detected in sera from rabbits inoculated with chronic lymphatic leukaemia lymphocytes, but this was directed against immunoglobulin itself. It is suggested that the membrane surface antigens of B cells are masked by immunoglobulin which is found on the surface of these cells as antigen receptor sites.

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