PROTEIN SYNTHESIS IN VITRO BY THE HUMAN BLOOD LYMPHOCYTE

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SUMMARY

The development of thought and knowledge concerning the lymphocyte is outlined in a survey of the literature, with particular reference to the place of this cell in the immunological mechanism.

The experimental materials and methods are described for:

(1) cotton filtration of human venous blood leucocytes to give suspensions of lymphocytes contaminated only by erythrocytes;

(2) 24-hour lymphocyte culture in Eagle's medium with ^{14}C-leucine;

(3) the measurement of the incorporation of ^{14}C-leucine into TCA precipitable protein by the lymphocytes *in vitro*;

(4) the qualitative analysis of the synthesized protein by the method of autoradiography of IEP patterns of the soluble protein of culture extracts, using autologous plasma as carrier protein.

Preliminary work in the development of the techniques showed that polymorph contamination of

human blood lymphocyte cultures influences the total protein synthesis *in vitro*, indicating the need to use lymphocyte suspensions uncontaminated by polymorphs. Some quantitative results using ^{12}C-leucine-free Eagle's medium are presented.

The characteristics of the culture system are described with respect to the cell population density, cell viability and the specific activity of ^{14}C-leucine in the culture medium. The contaminating erythrocytes did not significantly alter the total incorporation of ^{14}C-leucine into protein.

It is shown that the plasma-free culture system is suitable for short term (24 hour) lymphocyte culture, although the conditions are suboptimal. This system lends itself to the study of drug and hormone effects on lymphocytes *in vitro* in the absence of plasma binding.

By the use of metabolic inhibitors it is shown that the measured incorporation of ^{14}C-leucine represents true *de novo* protein synthesis. The significance of the incorporation of radio-isotope labelled amino acid precursor into protein is discussed with reference to the literature.

Investigation of autoradiographic labelling of IEP

patterns showed that labelling of the immunoglobulin lines and labelling in the albumin zone represents true de novo synthesis of the labelled components, while much of the labelling in the α_2-globulin zone to the anodal side of the origin is probably non-specific.

The results are presented of quantitative studies of total protein synthesis in vitro by blood lymphocytes from normal persons and patients with neoplastic lymphoproliferative disorders and infectious mononucleosis.

The presence of 25 per cent autologous plasma in the culture medium increased total protein synthesis by the lymphocytes in vitro. PHA stimulated total protein synthesis whether in plasma-free or 25 per cent plasma medium.

The literature relating to the effects of plasma and PHA on cell growth and metabolism in vitro is reviewed.

Total protein synthesis tended to be low in all clinical groups, except multiple myeloma, when the lymphocytes were cultured in plasma free medium, 25 per cent plasma medium and in plasma-free medium with PHA. In 25 per cent plasma medium with PHA the difference

between normal lymphocytes and lymphocytes from disease states was accentuated: under these conditions total protein synthesis by the lymphocytes was significantly low in CLL, lymphosarcoma, macroglobulinaemia and infectious mononucleosis.

The proteins synthesized in vitro by blood lymphocytes from normal persons and patients with neoplastic lymphoproliferative disorders and infectious mononucleosis were studied qualitatively by autoradiography of IEP patterns.

24-hour cultures of normal human blood lymphocytes showed labelling of α_2-globulins, β-globulins and immunoglobulins. Immunoglobulins were preferentially released into the plasma-free culture medium.

The presence of PHA or 25 per cent autologous plasma in the culture medium did not appreciably alter the pattern of labelling.

It was determined that in multiple myeloma the myeloma paraprotein was not synthesized by the blood lymphocytes. In Waldenström's macroglobulinaemia the blood lymphocytes synthesized macroglobulin, but not in excessive amounts.

In all cases of neoplastic lymphoproliferative disease there was immunoglobulin synthesis by the

blood lymphocytes *in vitro*. IgA and IgG were synthesized in most cases of CLL and lymphosarcoma. IgM was synthesized in most cases of CLL but there was a relative decrease in IgM synthesis in lymphosarcoma.

In some cases of CLL and lymphosarcoma the labelling of individual immunoglobulins in the autoradiographic patterns was increased or decreased (relative to the labelling of other precipitin lines) suggesting disordered immunoglobulin synthesis.

In Hodgkin's disease and reticulum cell sarcoma the overall immunoglobulin synthesis by the lymphocytes was not reduced. In certain cases of CLL, lymphosarcoma and unclassified 'lymphoma' the overall immunoglobulin synthesis was markedly reduced.

α_1 - lipoprotein labelling was detected in the majority of lymphocyte cultures from neoplastic lymphoproliferative disorders and infectious mononucleosis. This component was not labelled in cultures of normal lymphocytes, nor in cultures from other diseases.

The significance of these results, both quantitative and qualitative, is discussed in the light of the related findings of other investigators.