Mechanisms Involved in Cell-mediated Immunity

B. P. MACLAURIN, MB, MRCP, FRACP, Senior Lecturer in Medicine, University of Otago Medical School, Dunedin, New Zealand

Immunological aspects of diseases affecting the gastro-intestinal tract have received increasing attention in recent years with the demonstration of auto-antibodies against stomach, liver and colon constituents, and with emphasis on the importance of antibodies of the IgA class secreted into the bowel lumen. The relationship between intensive stimulation by antigens derived from bacteria and viruses and maturation of the immune response has also become evident from studies on germ-free animals; and the influence of bacterial flora upon the villous structure of the small bowel has been recognised (Abrams *et al.*, 1963).

However, relationships between the auto-antibodies demonstrated, and the histological abnormalities found in various liver and bowel diseases, remain uncertain; in most instances there is no proof that these antibodies are not the result rather than the cause of the disease. Because of the intense lymphocytic infiltration which is a feature of some bowel diseases, it has been suggested that cell-mediated immune damage of delayed type might be important in pathogenesis, e.g. in ulcerative colitis, and there is some experimental evidence to support this view (Perlmann and Broberger, 1963).

Studies on cell-mediated immunity in health and in gastro-intestinal disease have been in progress in our laboratory for the past seven years, and some of the results will be outlined in this article.

THE MIXED LYMPHOCYTE REACTION

The description by Pearmain *et al.* (1963) in New Zealand of antigen-induced lymphocyte transformation and proliferation stimulated an enormous volume of research on the mechanisms of lymphocyte activation. Bain *et al.* (1964) reported a special variety of lymphocyte activation induced by contact with allogeneic lymphocytes from an unrelated donor. This Mixed Lymphocyte Reaction (MLR) provides a useful *in vitro* model for study of receptor mechanisms in lymphocyte activation. It may also be an *in vitro* counterpart of homo-

graft rejection and thus have effector significance (Maclaurin, 1965a, 1966, 1967, 1968a).

The microscopic findings after mixed lymphocyte culture for five to seven days were compared with those at ten to fourteen days, with particular emphasis on cell-to-cell relationships. At the earlier time, temporary cytoplasmic connections were described between lymphocytes and macrophages, and lymphocytes and blast cells in rosette formations (Maclaurin, 1965b); such connections might permit ready transfer of informational material between different cell populations of the immune system (receptor mechanisms). It was postulated that the filamentous cytoplasmic connections observed between lymphoid cells after longer periods of mixed culture might have an attacking role (involving effector mechanisms in the cellular immune response).

More recently, in collaboration with Dr N. R. Ling of the Department of Experimental Pathology of the Birmingham Medical School, the author had the opportunity of studying a special category of the MLR in which human lymphocytes are cultured with irradiated lymphoma cells from a continuously maintained lymphoid cell line. In this test system an effector cytotoxic action of activated normal lymphocytes against the tumour cells has clearly been demonstrated (Hardy *et al.*, 1970). This test was applied to the lymphocytes of patients known to have an increased risk of developing lymphoid neoplasms, and statistically significant reduction of proliferative and cytotoxic capacity against tumour cells was found in coeliac disease (Maclaurin *et al.*, 1971a). Diminished cytotoxic capacity was observed for the lymphocytes of some patients with Crohn's disease (Maclaurin *et al.*, 1971b) and diminished proliferative capacity against tumour cell antigens for some patients with rheumatoid arthritis (Maclaurin, 1971a). These impaired responses appeared to be partly due to the presence of a depressive serum factor in all three patient categories, but evidence of a specific cellular defect in the lymphocytes from patients with coeliac disease was also obtained. These results may provide insight into the biological significance of the MLR in controlling neoplastic mutation.

RECEPTOR MECHANISMS STUDIED BY MACROPHAGE TISSUE CULTURE

Studies of the MLR highlight the close functional relationship between macrophages and lymphocytes and removal of macrophages from this test system diminishes the degree of response observed; it was also noted that the plant mitogen phytohaemagglutinin regularly altered the behaviour of macrophages as well as lymphocytes. It caused a rapid aggregation of macro-

phages maintained in monolayer culture and a striking increase in the frequency and duration of cytoplasmic bridging contacts between macrophages and between macrophages and lymphocytes added to the cultures (Maclaurin 1968b, 1969a). Human macrophages derived from Mantoux positive subjects showed similar aggregation and increased cytoplasmic contacts when exposed to tuberculin. It was therefore suggested that the increased cytoplasmic contacts between lymphoid cells induced by exposure to antigen might facilitate informational exchange, and the accepted role of the macrophage in antigen processing seems in line with this view.

Rabbit anti-rat thymocyte serum induces apparently identical cytoplasmic contacts between adjacent macrophages. Most of this macrophage specificity of anti-thymocyte serum could be removed by prior absorption with thymocytes, indicating the presence of common antigens on the surface of these two cell types. Electron microscopic study of cell to cell relationships between macrophages and lymphocytes after phytohaemagglutinin stimulation and after anti-thymocyte serum shows a well marked increase in the frequency of close cytoplasmic contacts between lymphoid cells, but no definite breaks in the continuity of the plasma membrane at these sites were found (Maclaurin and Humm, 1970).

ANTIGENIC AND FUNCTIONAL CHANGES IN THYMUS-DERIVED LYMPHOCYTES

The means by which the thymus confers the capacity to mount a normal cellular immune response has been intensively studied but with inconclusive results. One of the more interesting developments has been the demonstration that some mouse strains carry an extra antigen called theta, on thymus derived lymphocytes but not on bone marrow derived lymphocytes (Reif and Allen, 1964). By utilising the culture technique outlined in the previous section, it was shown that rat macrophages in monolayer cultures could distinguish between isologous and homologous lymphocytes of lymph node or of thymic origin, as judged by a significant increase in phagocytosis of homologous as compared to isologous cells, and of thymocytes as compared to lymph node lymphocytes (Maclaurin 1969b). The latter finding suggests that in the rat there may also be antigenic differences between lymphocytes from the thymus and cells derived from lymph nodes.

Recent work utilised two inbred rat strains, known to differ at the major AgB histocompatibility locus. Mixed lymphocyte cultures between pairs of animals of these strains, intact or following neonatal thymectomy, have strongly suggested a thymus origin for lymphocytes stimulating as well as reacting in mixed lymphocyte cultures (Maclaurin, 1971b). This finding could

indicate that the extra antigen on thymus passaged lymphocytes in the rat may modify the histocompatibility antigen display on the cell surface.

The view that the thymus may have a modifying influence on lymphocyte histocompatibility antigens is supported by some current studies on the activity of a thymic membrane-related ribonucleic acid preparation derived from and tested in the same inbred rat strains described above (Maclaurin and Gardner, 1971). This RNA preparation, injected in the neonatal period, appears capable of producing a temporary change in histocompatibility antigen characteristics, with prolongation of graft rejection times and change in MLR reactivity, not seen after spleen or liver RNA injection.

PRODUCTION OF AUTOIMMUNE HEPATITIS IN RATS

In attempting to assess the importance of lymphocyte mediated tissue damage in autoimmune disease, an animal model seemed desirable. By chronic immunisation of inbred rats with isologous or homologous liver homogenate in complete Freund's adjuvant, a portal triaditis was induced (Maclaurin and Humm, 1968). The lesion was accompanied by evidence of delayed hypersensitivity to a supernatant fraction of the crude liver homogenate and could be transferred to isologous animals by serial spleen cell injections from the immunised animals (Maclaurin, 1971c). However, there was also evidence of antibody formation, demonstrated by complement fixation and fluorescence microscopy, directed mainly against bile duct constituents. It was concluded that a true autoimmune hepatic lesion had been produced mainly as a result of a cell-mediated immune reaction. The histological changes in the liver appeared quite similar to those observed in human liver disease complicating ulcerative colitis. In addition, the directly immunised and transfer immunised animals showed intense peribronchial lymphocytic infiltration with partial disruption of the bronchial mucosa, features not found in animals treated only with Freund's adjuvant, or receiving serial transmission of spleen cells from non-immunised animals. The lung lesion might be the result of antigenic similarities between liver and lung since both structures are derived from entodermal outpouchings. It is of interest that pulmonary fibrosis has been reported in patients with chronic liver disease (Turner-Warwick, 1968).

CONCLUSIONS

It is suggested that the mixed lymphocyte tissue culture system provides a very useful model for investigation of some aspects of receptor and effector mechanisms in cell-mediated immunity. It has the advantages of simplicity, ease of observation at various stages in the reaction, and precise quantitation

(by measurement of tritiated thymidine incorporation into DNA). Furthermore, it is well suited to the study of human subjects because of the ready availability of blood lymphocytes. The special category of mixed culture using human lymphocytes with lymphoma cell line lymphocytes has the additional merit that the effector destructive function of stimulated lymphocytes can be precisely quantitated by measurement of ^{51}Cr release from labelled tumour cells. This technique is applicable to assessment of lymphocyte capacity in a wide range of human diseases, and studies of this type are currently in progress.

Acknowledgements

Most of the studies described were financed by a project grant from the New Zealand Medical Research Council. The remainder were carried out in the Department of Experimental Pathology of the Birmingham Medical School and acknowledgement is made to the Nuffield Foundation for the award of a Travelling Fellowship in Medicine, and to Professor P. G. H. Gell and Dr N. R. Ling for their support and encouragement.

References

Abrams, G. D., Bauer, H. and Sprinz, H. (1963) *Laboratory Investigation*, **12**, 355.
Bain, B., Vas, M. R. and Lowenstein, L. (1964) *Blood*, **23**, 108.
Hardy, D. A., Ling, N. R., Wallin, J. and Aviet, T. (1970) *Nature*, **227**, 723.
Maclaurin, B. P. (1965a) *Lancet*, **2**, 816.
Maclaurin, B. P. (1965b) *Lancet*, **1**, 1278.
Maclaurin, B. P. (1966) *Proceedings of the University of Otago Medical School*, **44**, 27.
Maclaurin, B. P. (1967) *Australasian Annals of Medicine*, **16**, 193.
Maclaurin, B. P. (1968a) *Bibliotheca Hematologica*, **29**, 602.
Maclaurin, B. P. (1968b) *Proceedings of the University of Otago Medical School*, **46**, 9.
Maclaurin, B. P. (1969a) *Australian Journal of Experimental Biology and Medical Science*, **47**, 105.
Maclaurin, B. P. (1969b) *Proceedings of the University of Otago Medical School*, **47**, 83.
Maclaurin, B. P. and Humm, J. A. (1968) *Proceedings of the University of Otago Medical School*, **46**, 75.
Maclaurin, B. P. and Humm, J. A. (1970) *Clinical and Experimental Immunology*, **6**, 125.
Maclaurin, B. P. (1971b) *Lancet*, **1**, 1070.
Maclaurin, B. P. (1971c) Submitted for publication.
Maclaurin, B. P. (1971a) *Gut*, in press.
Maclaurin, B. P., Cooke, W. T. and Ling, N. R. (1971a) Submitted for publication.
Maclaurin, B. P., Ling, W. T. and Cooke, N. R. (1971b) In preparation.
Maclaurin, B. P. and Gardner, J. (1971) In preparation.
Pearmain, G., Lycette, R. R. and Fitzgerald, P. H. (1963) *Lancet*, **1**, 637.
Perlmann, P. and Broberger, O. (1963) *Journal of Experimental Medicine*, **117**, 717.
Reif, A. E. and Allen, J. M. V. (1964) *Journal of Experimental Medicine*, **120**, 413.
Turner-Warwick, M. (1968) *Quarterly Journal of Medicine*, **37**, 133.