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MECHANISM OF ACTION OF ANTIGEN

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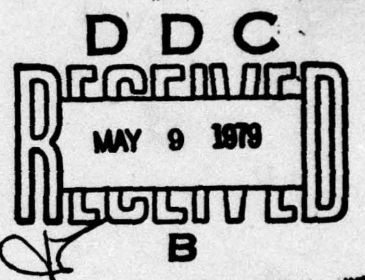

by

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During this research project on the mechanism of action of antigens, very significant progress on several fronts has been made. The results have met our expectations of this project and have clearly defined the significant questions for final resolution by future research in this area.

A major advance has been the quantitation of IgG and IgM immunoglobulins present in the plasma membranes of thymic lymphocytes which have been considered until now to lack membrane immunoglobulin. The import of this finding is that

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the evidence for thymic lymphocytes as antigen-reactive cells is consistent with the postulate of immunoglobulin as cell receptor for antigen. Evidence for surface immunoglobulin on thymic lymphocytes was lacking because of a) the failure of surface iodination to label more than a small fraction of thymic lymphocyte immunoglobulin and b) the markedly greater efficiency of iodination or peripheral lymphocytes compared to thymic lymphocytes. Thymic lymphocyte membranes are not as efficiently labeled as membranes from splenic lymphocytes and more specifically, IgG immunoglobulin in thymic lymphocytes is not labeled at all. We have concluded that IgG immunoglobulins are buried within the matrix of the plasma membrane and not accessible to external labeling reagents.

We have selectively extracted thymic lymphocyte glycoproteins using the lithium diiodosalicylate method. This method of extraction has been shown to be highly efficient for glycoproteins in particular and recent results have demonstrated that thymus specific antigen is enriched in these extracts. The extracted glycoproteins have been characterized and the method has been established as an efficient reproducible technique for the isolation and characterization of the rat lymphocyte antigens which are clearly associated with genetic control of immune responsiveness.

The delineation of differences between high responder and low responder lymphocytes is not being studied by in vitro biosynthesis of lymphocyte membrane components using incorporation of radioactive amino acids. We have found in thymus lymphocytes that two proteins of approximately 30,000 and 15,000 daltons rapidly incorporate amino acids and have a half-life of approximately 6 hours. These proteins are being identified by immunochemical methods already developed in the laboratory. The rates of synthesis and degradation of the thymocyte membrane IgG immunoglobulin are also being compared in high and low responder strains to define metabolic differences which correlate with in vivo immunological responsiveness. Whether or not these buried immunoglobulins function as antigen receptors requires labeling of this immunoglobulin to a very high specific activity with radioactive amino acids. Final resolution of this question will rest on future work based on this fundamental work supported by the Research and Development Command.

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The major objective of this contract was to investigate the mechanism of action of antigens. The experimental system that was chosen were inbred rats so that the genetic component as well as the immunochemical aspects of antigen action could be studied. The major problem was to find the interaction of the synthetic antigen poly($Glu^{52}Lys^{33}Tyr^{15}$) with lymphocytes from the highly responding ACI inbred strain of rats and from the poor responding F344 strain.

In the course of this work we isolated lymphocyte plasma membranes of high and low responder animals and analyzed biochemical and enzymatic composition. A thorough study showed that there were a few observable biochemical differences between the cell membranes from lymphocytes derived from the same organs in the high and low responder strains, but there were several major biochemical and enzymatic differences between lymphocyte membranes from the spleen and from the thymus: (a) lypid content is higher in thymus than in spleen; (b) carbohydrate content is slightly higher in spleen than in thymus; (c) sialic acid content in thymus is more than twice that in spleen; (d) 5'-nucleotidase specific activity is twice as much in spleen than in thymus in the case of the high responder strain (ACI), but this difference is absent in the case of the low responder strain(F344); (e) SDS-polyacrylamide gel electrophoresis analysis of the plasma membranes showed significant differences between the spleen and thymus with respect to their glycoprotein components. The thymocyte plasma membranes showed two major specific glycoproteins and we initially suggested that they represent thymocyte differentiation antigens. In our subsequent study with rabbit anti-rat brain anti-serum and rabbit anti-rat thymocyte antiserum, we proved that these two components do represent the thymocyte differentiation antigens.

In the course of these studies we also develop d techniques of quantitating immunoglobulins in the lymphocyte plasma membranes and metabolic techniques for studying the events initiated by the reaction of antibody and antigen. We were able to demonstrate clearly that lymphocytes from both the thymus and the spleen have IgM and IgG molecules in their plasma membranes. Approximately 1% of the membrane protein of thymocytes is IgG immunoglobulin. By surface labeling, only trace amounts of IgM were detected on thymic lymphocytes. These findings led us to conclude that IgG immunoglobulins are buried within the matrix of plasma membranes and not accessible to external labeling reagents in thymocytes. On the contrary, immunoglobulins are accessible on the surface of splenic lymphocytes. These investigations provide clear evidence for settling a major argument in the immunological literature.

As a second approach to demonstrate the presence of IgG and IgM in thymocyte membranes, we studied the biosynthesis of these molecules by two different cell types. We were able to show that splenic lymphocytes synthesize and secrete both IgG and IgM whereas thymocytes secreted only IgG. The synthesis of IgG increased in the thymocytes after immunization, whereas the biosynthesis in splenic lymphocytes was unaffected. These immunoglobulins were isolated in a supernatant fluid of stimulated and non-stimulated thymic lymphocytes. In addition, stimulation with large amounts of antigen can induce tolerance and decrease the amount of immunoglobulin synthesized by these lymphocytes.

Thus, we have been able to achieve three major goals: 1) the demonstration of IgG and IgM in thymic lymphocytes as well as splenic lymphocytes, 2) a detailed analysis of the protein and glycoprotein structure of lymphocytes and, 3) demonstration of the biosynthesis of IgM and IgG by splenic lymphocytes and IgG by thymic lymphocytes.

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