The Significance of the Carrier Effect for the Induction of Antibodies

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Introduction

When an immunogen is injected into a higher organism, antibodies reacting specifically with that immunogen are produced. Since a vast number of antibody specificities exist, and since cogent evidence indicates that antibody specificity is determined by the primary structure of the antibody polypeptide chains, the question has to be asked of how antigen induces the structural genes coding for the "right" antibodies, and only these, to initiate protein synthesis.

Upon immunization with an antigen, only a small proportion of the population of immunocompetent cells present in the organism starts to multiply and to produce specific antibodies. Strong evidence supports the idea that for any given antigen a specific subpopulation of immunocompetent cells exists, which is antigen-sensitive, i.e. which can be stimulated by that antigen to produce specific antibodies. If a given immunocompetent cell has the capacity to produce antibodies of just one or very few specificities, the problem of antibody induction is reduced to the question of how antigen specifically stimulates those cells that are able to produce an antibody fitting to that antigen. The simplest way of specific stimulation would be the presence of specific receptors for antigen on the surface of the immunocompetent cells. Since these receptors must have the same range of specificities as the specific product of the cells, e.g. the antibodies, it is straightforward to believe that the postulated receptors for antigen on the cell surface are antibody, — that antibody, that a given cell is capable to produce. This in fact is just a restatement of Jerne's original idea (Jerne, 1960) that antigen recognition must occur via specific antibodies. Though direct evidence for cellular immunoglobulin receptors of the type described here is lacking, there is strong evidence favouring this view (Mitchison, 1969 b).

The induction process would be based, then, on combination of a given antigenic determinant with a specific receptor on a specific immunocompetent cell. We are going to describe in this article recent experiments which point to a more complex mechanism of antibody induction.

The Carrier Effect: Cooperation of Antigenic Determinants

We will discuss here the evidence showing that the induction of specific antibodies by an antigen requires the recognition of more than one determinant of the antigen. This implies that immunogenic substances must possess at least two antigenic determinants.
The work of Landsteiner (1947) has established that in order to be immunogenic, simple chemical compounds have to be coupled to a macromolecular carrier. Substances that are unable to induce an immune response by themselves, but which can be recognized by antibody, were designated as haptens. What is the role of the carrier in rendering a hapten immunogenic?

It has long been known that there is some specificity associated with the role of the carrier: An animal primed with hapten on carrier A responds poorly or not at all to a secondary injection of the hapten on carrier B, whereas injection of the hapten on carrier A leads to a good secondary response. Since antigenic determinants are frequently composed of the hapten and some of the adjacent carrier surface, this finding does not a priori need an elaborate explanation. Recent studies indicate, however, that the role of the carrier cannot be fully understood on the basis of a "local environment hypothesis" (see Mitchison, 1967). Hapten-carrier systems have been described where hapten and carrier are macromolecules of similar size. As shown by Benacerraf and his coworkers (Benacerraf, et al., 1967), DNP-polylysine (DNP-PLL; molecular weight approximately 60,000) is non-immunogenic in a certain strain of guinea pigs. The same guinea pigs respond, however, to immunization with DNP-PLL complexed with foreign serum albumin with the formation of large amounts of antibodies to serum albumin and to the DNP determinant. The five lactic dehydrogenase (LDH) isozymes are tetrameric molecules composed of two types of subunits (A and B) in all possible combinations (for review see Kaplan, 1964). It was found (Rajewsky et al., 1967; Rajewsky and Rottlander, 1967; Armerding and Rajewsky, 1969) that a certain line of rabbits was unable to fully respond to the B4 enzyme, whereas a normal response occurred upon immunization with the A4 enzyme. If the animals were immunized with the B2 A2 hybrid, however, similar amounts of anti-A and anti-B antibodies were formed. In both systems, antibodies to the haptenic macromolecule did not show any detectable reaction with the carrier.

The role of the carrier was specific in both systems: DNP-PLL complexed with guinea pig serum albumin proved to be non-immunogenic. And porcine haptenic B-subunits hybridized with (carrier) A-subunits from rabbit were unable to induce a secondary anti-B response in rabbits primed with the porcine A2 B2 hybrid. Perhaps most striking, it could be shown in both systems that induction of tolerance to the carrier led to a reduced response to carrier and hapten upon immunization with the hapten carrier complex. Quantitatively, the reduction of the response was similar in this situation for anti-hapten and anti-carrier antibodies, as demonstrated in the LDH system (Fig. 1).

Thus, in both the LDH and the DNP-PLL-BSA system, it appears unlikely that the carrier only completes the antigenic determinants of the hapten. Instead, antigenic determinants of the hapten and those of the carrier seem to cooperate in some way in the induction of the immune response to the hapten carrier complex. Mitchison, who has clearly stated this idea of cooperating antigenic determinants, came to the same conclusion in his conventional hapten carrier system. He showed, that for the induction of the secondary response, the hapten could be separated from the carrier by a spacer group without abolishing the carrier effect (Mitchison, 1967). Further evidence supporting the cooperation concept came from the work of Schierman and McBride (1967) in their system of chicken isoantigens. Recent