SPELULATIONS ON THE FUNCTION OF IMMUNE RESPONSE GENES

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SUMMARY

Immune response (Ir) genes linked to genes encoding histocompatibility antigens affect antibody-formation to synthetic polypeptides, weak native antigens and strong native protein antigens given in limiting doses. It has been proposed that the products of such Ir genes function as receptors for antigen on thymus-derived lymphocytes (T-cells) and these products are not immunoglobulin in nature. However, recent evidence indicates that (a) Ir genes are probably expressed in both T-cells and in bone-marrow-derived lymphocytes (B-cells); (b) IgM immunoglobulin is present on T-cells and functions in the binding of antigen, and (c) T-cells of nonresponder animals possess the capacity to recognize antigen. These results prompted us to consider alternative hypotheses for the function of Ir genes; namely (1) 'tolerance' hypotheses in which response is dominant over non-response, and (2) the possibility that the Ir gene product can act as an amplifier of T-cell function. These hypotheses are discussed within the context of murine studies and in relation to the association of human diseases, notably immunopathic phenomena, with HL-A.

INTRODUCTION

The immune response to a variety of antigens including synthetic polypeptides and native antigens is governed by autosomal dominant genes. A number of such genes exist, but the Ir-1 system of mice (McDevitt & Sela, 1965) and the PLL gene of guinea-pigs (Benacerraf et al., 1967) have been studied in greatest depth. These two systems share properties which throw the problem of the relationship between products of immune response genes and lymphocyte surface receptors for antigen into sharp relief. Inasmuch as the characteristics of Ir gene effects have recently been extensively reviewed (Benacerraf & McDevitt, 1972; McDevitt & Landy, 1972), we will summarize the present consensus rather than give a
detailed analysis of the data underlying the conclusions. The properties of the Ir-1 and PLL systems which are germane to this discussion are as follows: the genes are (a) autosomal dominants which are (b) linked to major histocompatibility antigen genes and (c) expressed in thymus-derived lymphocytes (T-cells). Furthermore, the effect of these genes on the immune response is (d) correlated with particular antigenic specificities. For example, in mice the capacity to mount a γG response to a synthetic branched polymer of L-tyrosine, L-glutamic acid, D,L-alanine, and L-lysine termed (T,G)-A-L is affected by a gene located between the H-2D and H-2K histocompatibility loci (McDevitt et al., 1972). Individuals of a responder strain (e.g. C57BL/6) can produce γM and γG antibodies and show immunological memory to this antigen, whereas members of a 'nonresponder' strain (e.g. CBA) can manifest a similar γM primary response but cannot form γG antibodies or show a secondary response.

Much of the discussion concerning certain immune response (Ir)* genes has been based upon the presumption that the products of such genes must function as receptors for antigen on thymus-derived-lymphocytes (T-cells). Subsequent argument among those accepting this conclusion consisted of debate whether these products were immunoglobulins, or whether they represented some new immunological recognition molecule (McDevitt & Landy, 1972). Although the 'receptor hypothesis' for Ir gene products is attractive in many ways, including simplicity, data from a variety of sources suggest that it may not be the correct explanation of Ir gene function. In this paper, we will review these arguments and then outline two alternative hypotheses of Ir gene function which are permitted in the context of present data. The first proposal represents a class of explanations which may be termed 'tolerance' models in which the non-responding animals possess a normal component which cross-reacts immunologically with the antigen in question. One particular model of this class raises the possibility that Ir genes are not structural genes, but regulatory genes, governing the synthesis or expression of the cross-reacting self component. The second model presumes that the products of Ir genes are amplifiers of T-cell response and that the observed specificity for antigen arises from clonal association of particular Ir products and immunoglobulin receptors in differentiated lymphocytes.

**IR GENE PRODUCTS AS ANTIGEN RECEPTORS ON THYMUS-DERIVED LYMPHOCYTES**

The fact that Ir genes showed specificity for antigen and were expressed on T-cells suggested that the products of such genes were most probably T-cell receptors for antigen (Benacerraf, 1972; Grumet & McDevitt, 1973). Moreover, the linkage of the Ir-1 gene to H-2 antigen loci and its apparent lack of linkage to known immunoglobulin genes (McDevitt et al., 1972) prompted the hypothesis that these receptors must differ from immunoglobulins which are currently accepted as the antigen receptors on bone-marrow-derived lymphocytes (B-cells), those lymphocytes which are the direct precursors of antibody-forming cells. This line of argument was buttressed by conclusions from indirect experiments that immune responses mediated by T-cells alone possessed a 'different specificity' or 'poorer binding' (Grumet &

* The term 'Ir gene' will, in this paper, designate immune response genes which are linked to major histocompatibility antigen genes, e.g. the Ir-1 locus of mice. Certain immune response genes are linked to immunoglobulin genes (Blomberg et al., 1972) and unquestionably encode receptor specificity.
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McDevitt, 1973) than that characteristic of B-cell products (antibodies), and certain workers claimed that the level of immunoglobulin on T-cells was so low as to be undetectable by extremely sensitive techniques (Unanue et al., 1971; Vitetta et al., 1972).

We would emphasize that all of these points are subject to some debate and serious experimental gaps occur in present knowledge, e.g. attempts at comparison of recognition specificity of T-cells and B-cells have been carried out with indirect biological assays; they have not been performed by measurements of antigen binding or the immediate biological consequences of cell exposure to antigens. Cohn (1972), for example, raised the possibility that the linkage of certain Ir genes to genes specifying histocompatibility antigens might have arisen fortuitously and not provide any direct insight into the function of such genes. The situation could represent an example of a 'frozen genetic accident' as described by Ohno (1973) where certain mammalian linkage groups represent historical accidents, rather than functionally or structurally related units. The map position of the Ir-1 locus in between the H-2D and H-2K genetic regions represents a situation which might have arisen via gene duplication and inverted translocation of the H-2 prototype gene (Schreffler et al., 1972). Such a linkage combination is ideally suited to 'freeze' loci which lie between the translocated pair.

Furthermore, recent evidence has accrued which forces us to question the role of the Ir gene product as a primary receptor for antigen. The requirement to invoke a new molecule as the T-cell receptor for antigen has recently become less obvious because numerous workers have shown that binding of antigens by T-lymphocytes can be inhibited by anti-immunoglobulin sera (Ashman & Raff, 1973; Dwyer et al., 1972; Hogg & Greaves, 1972; Marchalonis et al., 1973; Rutishauser & Edelman, 1972; Roelants et al., 1973; Lawrence et al., 1973). Moreover, the capacity of surface immunoglobulin isolated from activated T cells to combine specifically with the antigens used in the activation process has been directly demonstrated (Cone et al., 1972; Marchalonis & Cone, 1973; Feldmann et al., 1973; Röllinghoff et al., 1973). This surface immunoglobulin is characterized by a mass of approximately 180,000 daltons and is comprised of light chains and μ-like heavy chains (Marchalonis & Cone, 1973; Feldmann et al., 1973). It differs from surface immunoglobulin of B-cells in its cytophilic properties (Cone et al., 1973) and capacity to function as a collaborative factor in specific co-operation of T-cells and B-cells (Feldmann et al., 1973; Rieber & Riethmuller, 1973). Another area of contention directly raises the possibility that the Ir product is not the T-cell receptor for antigen. Gershon and his colleagues (1973) have presented evidence that thymus lymphocytes of nonresponder mice possess the capacity to recognize the proper antigen and respond by blast transformation. However, the thymus lymphocytes of nonresponder

† The original studies of Eisen (1959) indicated that cells mediating delayed-type hypersensitivity (T-cells) showed a greater recognition specificity than did antibodies (B-cell products), possibly because the T-cells possessed receptors capable of 'seeing' a larger portion of the antigen molecule. In recent usage, the concept of differences in recognition specificity of T-cells and B-cells has often been taken to imply that T-cells are less specific or show a 'broader specificity' than do B-cells (see Grumet & McDevitt, 1973). T-cells, in point of fact, show direct binding specificity for a number of antigens including haptenes (Lawrence et al., 1973; Rolley & Marchalonis, 1972; Möller et al., 1973) and synthetic polypeptides (Roelants et al., 1973; Schlossman, 1972). It is conceivable, however, that T-cell receptors possess a different library of recognition structures (V-regions) than do B-cells; i.e. their receptors may be complementary to different portions of the antigen and express different affinity constants than do B-cell produced antibodies. These differences do not necessarily disqualify immunoglobulin as the primary T-cell receptor for antigen (vide infra). Moreover, observed specificity differences in T-cell and B-cell responses may involve susceptibility to activation or tolerization rather than recognition and primary binding of antigen.
animals were easily tolerized by contact with this antigen, whereas thymus lymphocytes of responder mice were not rendered tolerant. A third point which brings the receptor role of the products of H-linked Ir genes into question arises from studies which indicate that such genes are expressed in both B-cells and T-cells. Shearer et al. (1972) have shown by limiting dilution analyses that Ir-1 nonresponder mice to (T,G)-A-L are deficient with respect to responders at both the T-cell and B-cell levels. Furthermore, Katz et al. (1973) observed that primed T-cells of responder mice could not collaborate with B-cells of nonresponder mice, thereby raising the possibility that the Ir gene in question was expressed in both B-cells and T-cells. Moreover, Cheers & Sprent (1972) have shown that B-cells will act as targets in the initiation of a mixed lymphocyte reaction. This evidence is less direct than the above but is suggestive because the gene regulating the mixed lymphocyte reaction (MLR) is extremely closely linked, if not identical, to the Ir-1 locus (Shreffler, 1972; Bach, 1972). The argument proposing that the Ir gene product is a unique T-cell receptor for antigen becomes muddled because of the likely expression of this structure of B-lymphocytes.

In order for the product of the Ir gene to serve as receptor for antigen under these strictures it must be immunoglobulin; a conclusion which is consistent with Cohn's reasoning (1972). Even in this case, however, an additional factor is required because the second condition showed that recognition alone was not sufficient to generate a complete response (Gershon et al., 1973; Watson et al., 1973; Schrader, 1973). At this stage of investigation, there is no direct evidence for receptor function of products of H-linked Ir genes, but direct evidence (vide supra) exists that they are not involved in primary union with antigen. Analysis of the mechanism(s) of Ir gene regulation of antibody production will be aided materially when the products have been isolated and analysed. For the remainder of this paper we will consider alternative hypotheses for the function of immune responder genes.

TOLERANCE MODELS

One class of hypothesis which might explain the nature of Ir type genes is based upon the premise that nonresponder strains possess a cell surface component which is antigenically related to the test antigen. Since this is a normal self component the animal is tolerant to it and may also be tolerant to the related antigen. Experimental evidence consistent with this notion was obtained by Ebringer & Davies (1973) who reported that histocompatibility antigens of nonresponder mice (CBA) cross react with (T,G)-A-L. Moreover, work of Gershon et al. (1973) cited above suggests that the lesion in response of nonresponders to the synthetic polypeptide antigen comprized of glutamyl, alanyl and tyrosyl residues (GAT) is not involved in recognition of antigen but in a more readily-induced tolerance.

The major argument against 'tolerance' models is that non-response, rather than response, should be dominant since heterozygotes would still produce the tolerogenic component. This contention presumes that the Ir gene must be a structural gene encoding the self component. If, however, the Ir gene is either (a) a regulatory gene, (b) a gene encoding a factor which somehow masks the expression of the self antigen (e.g. an enzyme which degrades it or modifies it by addition of chemical groups), or (c) a gene controlling the function of 'suppressor' T-cells, situations where response was dominant could result. In cases (a) and (b), the heterozygotes (Ir+, Ir-) would respond because no (or little) self tolerogen would be present. Only the homozygous nonresponder case (Ir-, Ir-) would possess the tolerogen and be incapable of response.
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A diagram illustrating case (a) is given in Fig. 1. This represents the now classical Jacob-Monod operon concept (1961) but the elements are identified with a possible mechanism of Ir gene function. Another mechanism giving a comparable result will be described below. The Ir gene in this case is the regulatory gene which governs the production of a product which represses the function of the operator gene (O). When the allele (Ir+) encoding the functional repressor is present, the operator gene is turned off and no cross-reactive self product (ST) is formed. This situation obtains for the homozygous case (Ir+, Ir+) as well as the heterozygous configurations. The ST product is made only in the homozygous non-responder (Ir−, Ir−). This model is presented as an ‘all-or-none’ system; however, quantitative effects may be observed in the heterozygote with the result of ‘partial tolerance’ or ‘partial response’. Such observations have been made with allophenic or tetraparental mice comprised of responder and nonresponder genomes which should be completely tolerant but exhibit a variety of intermediate effects (Harris & Warner, unpublished observations).

![Operon-type model for action of immune response genes.](image)

Fig. 1. Operon-type model for action of immune response genes. O, operator gene for the ST structural gene; ST, structural gene encoding the self antigen (tolerogen) which cross reacts with the test antigen in question.

The second model of dominant response through tolerance considers that the self-tolerogen (ST) is always synthesized, but that another gene (Ir) exists which masks its presence in responders. One way this might be carried out is to presume that the Ir product possesses binding specificity for particular sites on ST and occludes it from immune surveillance. Another possibility is that the Ir product is an enzyme which specifically degrades or modifies ST. Such a situation is readily feasible, particularly if ST contains carbohydrate in its antigenic moiety, because glycosidases and specific glycosyl transferases are associated with cell membranes (Roth et al., 1971). In either case, homozygotes (Ir+, Ir+) and heterozygotes (Ir+, Ir−) possess this blocking agent and are phenotypically responders. Animals lacking the blocking agent or possessing an altered form (Ir−, Ir−) exhibit the self-tolerance factor and are phenotypically non responders.

The ‘suppressor’ T-cell case is formally similar to that developed above but entails further complexity because models based upon this approach involve regulator cells by regulator
genes. If the Ir gene product shuts off suppressor T-cells, effector T-cells would be allowed to function and the animals would be phenotypically responders. The only genotype in which suppressor T-cells would be operative is the homozygous Ir-case, which would consequently give a nonresponder phenotype. Examples of suppressor T-cells mediating 'infectious tolerance' to antigens such as sheep erythrocytes are well-documented (Gershon & Kondo, 1971; McCullach, 1973). However, the process or processes which establish tolerance to self components are those which appear most germane to the present discussion. Harris and Röllinghoff have recently carried out studies of tetraparental mice (personal communication) which provide no evidence for the action of suppressor T-cells in the generation of tolerance to allo-antigens within these animals. For this reason, it is not clear at this time whether suppressor T-cells are directly involved in the type of tolerance required in this model of Ir gene function.

**IR GENE PRODUCTS AS AMPLIFIERS OF SPECIFIC T-CELLS**

The general type of model we will discuss considers the possibility that the products of Ir genes function as amplifiers of T-cell function. This hypothesis gains support from studies of association between human auto-immune diseases with major histocompatibility loci to be discussed below. Moreover, experiments of two types have shown that nonspecific effects can transform nonresponding animals into responders. In the case of nonresponsiveness related to the Ir-1 gene the establishment of a graft-versus-host reaction brought about this transformation (Ordal & Grumet, 1972). In responses governed by the Ir-3 gene, injection of a synthetic polynucleotide adjuvant consisting of complexes of polyadenylic acid and polyuridylic acid (poly A:U) had the same effect (Mozes & Shearer, 1972). The latter experiment is of interest because poly (A:U) exerts a stimulatory effect on T-cells. It does not replace T-cells, but amplifies the effects of relatively small numbers of them such that an enhanced response can be achieved (Cone & Johnson, 1972; Cone & Marchalonis, 1972). For example, treatment of neonatally thymectomized mice with this adjuvant enables such animals to give a response to sheep erythrocytes comparable to that exhibited by normal mice. We would conclude from these findings that nonresponder mice need not lack T-cells specifically reactive to a given antigen. They may possess such cells in the low frequency normally expected from clonal restriction of specific antigen reactivity but be deficient in a mechanism for amplifying the number of these cells to a level where detection of specific immune reaction becomes feasible. Responders could possess equally small numbers of specifically reactive T-cells, but have the capacity to expand these clones rapidly.

Combining the conclusions that (a) immunoglobulin most probably serves as the T-cell receptor for antigen and (b) the Ir system serves to amplify the effects of relatively small numbers of specific T-cells with the above properties of the immune responder gene systems which are linked to histocompatibility antigens, it is possible to construct a model for the functional interaction between Ir gene product(s) and immunological receptor(s) in the genetic control of immune responses. For the purposes of the present argument, it is unnecessary to know the precise molecular nature of the Ir gene product, except to state that it is most likely a protein and it does not require primary combining specificity for the antigen in question. This protein could occur on the lymphocyte surface in close proximity to the immunoglobulin which functions as receptor for antigen. One of a number of possible
mechanisms is that the Ir gene product can recognize, not the antigen, but a particular conformational isomer of the immunoglobulin which results from its combination with antigen. The molecule would, thus, recognize the structure or a membrane conformational change formed by the union of antigen with antibody. Since antigens and, presumably, combining sites involving different V-region sequences, differ in steric and energetic properties, a set of Ir products might be necessary to recognize the possible variety of site-antigen conformations. Another possibility is that recognition by Ir products is associated, not with complementary recognition effected by a small portion of a macromolecule such as the antigen combining site of immunoglobulins but with more widely distributed surface properties of molecules or cells such as surface charge. A second property of the Ir product is that in order to activate other cells it must possess the capacity to recognize complementary structures which occur on the surfaces of T-lymphocytes (and possibly B-lymphocytes) and combine with this molecular configuration. When this latter event occurs, cells which bear the proper receptor are activated to proliferate and produce more Ir gene product as well as the surface immunoglobulin which they express.

The minimal means of achieving this recognition and cell activation is to propose that the polypeptide encoded by a particular Ir gene has a conformation which allows it to combine with other molecules specified by the same gene and form specific oligomeric aggregates. Such specific polymerization might be correlated with conformational changes in both parties. If one monomer were associated with the cell membrane at the time of combination with a monomer free in solution, the energy of the conformational change might stimulate the lymphocyte to proliferate. In the resting lymphocyte, only a small amount of Ir product would be synthesized and this would be associated predominately with those lymphocytes forming it. After stimulation by either antigen or Ir product (vide supra) large amounts of Ir product would be secreted. The Ir gene product can, thus, be considered a mitogen which affects lymphocytes and would possess a dominant inheritance pattern. The concept of T-cells giving both a specific response and producing nonspecific factors which augment responses to other antigens is supported by data of Waldmann et al. (1973). T-cells reactive to proteins can reconstitute the response of T-cell depleted spleen cell cultures to non-cross-reacting antigens. This effect was obtained only if the initial activating antigen was present in the culture. The authors argued that the T-cells did not merely produce a general activator of T-cells because the background level of cells reacting to sheep erythrocytes was not increased. However, this result resembles the situation produced during amplification of T-cells with poly (A:U). The antigen must be present with the adjuvant in order for amplification of response to that antigen to occur.

Since at least three distinct, but closely linked, Ir loci occur in a chromosomal region between the H-2K and H-2D loci which is sufficiently large to contain over 100 genes (Klein & Shreffler, 1971; McDevitt & Landy, 1972), it is not unreasonable to postulate that a family of Ir products exists. These polypeptides would be closely homologous because of a common evolutionary history but differ from one another sufficiently to decrease or eliminate binding of products of distinct Ir genes to one another. A necessary concomitant of this situation is that the receptors for Ir products (themselves Ir products) which occur on lymphocytes of a population vary from individual cell to individual cell. Thus there exists a set of Ir products, each one encoded by a germ line gene. This set need not be extremely large; 100 genes would be more than sufficient to account for observed results and is consistent with the amount of space available to Ir genes within the H-2 region of the chromosome.
CLONAL SELECTION OF IR GENE PRODUCTS AND IMMUNOGLOBULIN RECEPTORS

Fig. 2 illustrates the clonal nature of the effect of a particular Ir product on a differentiated lymphocyte population. The phenotypic restriction of lymphocytes in terms of clonal selection of immunoglobulin receptors (Burnet, 1959) also provides the means for associating the presence of a particular Ir product with the capacity to respond to particular antigens. Immunologically competent lymphocytes develop in ontogeny from multipotential stem cells. Mature lymphocytes are restricted in their capacity to respond to antigen. A given cell exhibits a specific receptor for one antigen, and combination with that antigen triggers immune differentiation. For present purposes let us consider a set of immunoglobulin receptors comprised of at least 10,000 elements corresponding to the variable regions of light chains or heavy chains. This set can be represented: \( a_1, a_2, a_3 \ldots a_m \) where the individual proteins differ from one another in amino acid sequence correlated with the capacity to form combining sites for different antigens. The set of Ir gene products could consist of less than 100 elements, the members of which are also clonally distributed throughout the lymphocyte population. The distribution of Ir products and Ig receptors can be random. If for example, the frequency of a particular Ig is \( 10^{-4} \) and the frequency of a particular Ir product is \( 10^{-2} \), the combined frequency of the two markers is \( 10^{-6} \). At a given time, a mouse thymus containing \( 10^8 \) cells would contain 100 cells expressing both proteins. A nonresponder strain would possess Ig receptors with the proper specificity, but the Ir gene product corresponding to this receptor would be absent or non-functional. T-cells of these animals could therefore recognize the antigen, but would be unable to proliferate to the degree required to initiate strong T-cell responses or trigger a B-cell response.

The functional association between a particular Ir product, e.g. \( I_{39} \), and a particular immunoglobulin combining site, e.g. \( a_{666} \), would manifest itself during the differentiation of
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lymphocytes in the developing animal. At some stage of the development of immunocompetent lymphocytes, the cells might express the Ir product and the capacity to synthesize the corresponding Ir product. Once this commitment has been made, all the progeny of that particular cell express the same Ir product. If the event regulating the commitment to a particular immunoglobulin receptor occurs simultaneously with or following the Ir-commitment, a clone of lymphocytes results which shows specificity for antigen and can be made to proliferate by contact either with Ir product or by the proper antigen. A small amount of a potent antigen, for example, would trigger a few specific T-cells. This specific stimulus would be amplified by a cascade effect in which the Ir product released by these cells would trigger a large number of the cells bearing the proper Ir product. A small antigenic challenge augmented in this manner could, thus, cause the activation of virtually all the members of a particular clone of reactive lymphocytes and, furthermore, accelerate the generation of more reactive cells by division. This concept is illustrated in Fig. 3. Since all cells bearing the proper Ir product can be stimulated, a large amount of cell division could occur and many of these cells would possess specific receptors for other antigens. This conclusion is consistent with the marked proliferation of lymphocytes which occurs during the initiation of cellular immune reactions (Turk, 1967) despite the fact that the frequency of cells with receptors specific for the challenging agent is usually low.

The preceding discussion has considered only the mitogenic effect of Ir-product on T-lymphocytes. It is conceivable that this product might interact also with B-lymphocytes. In this case it could serve as a second signal (i.e. one in addition to antigen (Bretscher, 1972), regulating the switch-over from synthesis of \( \gamma M \) antibodies to synthesis of \( \gamma G \) antibodies.

We cannot claim that this double clonal selection model will stand the test of time and deeper analyses into genetic relationships among Ir genes and immunoglobulins and into the chemical properties of Ir gene products. The present system is consistent with the properties characteristic of T-cell expressed Ir genes and, furthermore it is founded upon experimentally-falsifiable premises. We would predict that analyses of antigen-binding T-cells in responder and nonresponder animals would disclose low, but equal, numbers of such cells in both strains. After immunization, responders would show a large increase in the frequency of binding cells; nonresponders would be characterized by a marginal increase.

Fig. 3. Cascade effect of Ir product on proliferation of antigen specific T-lymphocytes which are committed in respect to immunoglobulin receptor as well as Ir product.
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The term 'nonresponder' is, thus, a misnomer because the differences would be quantitative, rather than absolute. The second major correlate of this model is that agents which non-specifically amplify T-cell function, e.g. poly (A: U) or allogeneic factors, should cause nonresponders to behave as responders if the Ir gene defect affects T-cells alone. A further consequence of the present model is that Ir gene product alone would not convert a nonresponder into a responder because lymphocytes bearing the Ir-product would be absent.

RELEVANCE OF THESE MODELS TO THE STUDY OF HUMAN HISTOCOMPATIBILITY SYSTEMS AND DISEASE

During recent years associations have been sought between HL-A, the major histocompatibility system in man, and disease susceptibility, stimulated in the first instance by the description of an association between susceptibility to the Gross virus leukaemia and H-2 in the mouse (Lilly et al., 1964). These studies have exploded into a torrent more recently following the definition of Ir genes linked to major histocompatibility systems in the mouse and guinea-pig. These studies have been reviewed in detail recently (Morris, 1973), but some of the more striking associations between HL-A and disease are relevant to the models of Ir gene products that we have postulated in this paper.

The most salient group of associations that exist with HL-A are those with certain immunopathic disorders. The most attractive explanation for these associations is based on linkage between HL-A and an immune response gene. However there is a difficulty in reconciling the observed associations with the more accepted concept of an Ir gene, which implies that the gene product is the receptor for antigen on T-cells and therefore exhibits a great degree of specificity. The difficulty lies in the observation that the following immunopathic disorders, systemic lupus erythematosus (Waters et al., 1971; Grumet et al., 1971; Morris & Mackay, 1973), active chronic hepatitis (Mackay & Morris, 1972), thyrotoxicosis (Grumet—personal communication; Whittingham, Martin & Morris—unpublished), myasthenia gravis (Engelfriet et al., 1973) and adult coeliac disease (Stokes et al., 1972; Falchuk et al., 1972), have shown a very strong association with one antigen, namely HL-A8. In addition less convincing associations with HL-A8 have been shown with childhood asthma (Thorsby & Lie, 1971) and renal allograft rejection (Kissmeyer-Nielsen et al., 1971). Thus there appears to be a lack of specificity and possibly an enhanced reactivity in these associations which is not compatible with the demonstrated Ir gene responses in the mouse. However, it must be borne in mind that these Ir gene associations in experimental animals have been demonstrated in inbred strains with selected antigens. These findings may not be strictly analogous to the situation in the outbred population of man, where his immune responses have been modified no doubt by selective pressures in the course of evolution. We feel that our model of Ir gene products acting as an amplifying system might explain the association of such apparently different immunopathic disorders as active chronic hepatitis and coeliac disease with HL-A8. For, as mentioned previously, the clonal distribution of a limited number of Ir products through the lymphocyte population might result in the same Ir product being associated with the receptor for a number of different antigenic receptors concerned with the immunogenic stimulus in the above disorders.

If this particular Ir gene were linked with the gene for HL-A8 then this could explain the association of HL-A8 with a number of different immunopathic disorders. Again if any selective advantage could be attributed to the Ir product associated with HL-A8, this could
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lead to a relatively high gene frequency of that particular Ir gene relative to other Ir genes in what is presumably now a balanced polymorphism.

Other HL-A and disease associations might be better explained, if one is to attempt to attribute all such associations to linkage of HL-A with Ir genes, on the basis of one of the postulated tolerance models. This is particularly true of the possible viral induced diseases such as the leukaemias and poliomyelitis (Morris, 1973). However, this is on less firm ground than the association between HL-A and the immunopathic disorders where we feel that the amplification model can explain very readily the observed associations.

CONCLUSIONS

We considered the problem of the manner in which Ir genes linked to genes encoding histocompatibility antigens might function in the control of immune responses to certain antigens. The most direct hypothesis was that the products of Ir genes, in particular Ir-1 genes of mice, were receptors for antigen on T-lymphocytes. This proposal was discussed at length in a recent symposium (McDevitt & Landy, 1972) and it was concluded by Cohn that if this hypothesis, in fact, were true the Ir products would either be κ-light chains or possibly a subclass of μ-type heavy chains. The opposing school of thought, led by Benacerraf & McDevitt, agreed that Ir products were T-lymphocyte receptors for antigen, but proposed that these molecules were not immunoglobulin, but some new class of immunological receptor. In this paper, we discussed recent evidence which supported Cohn’s conclusion. Moreover, consideration of these data forced us to consider other possibilities for Ir gene function in addition to that of encoding antigen receptor molecules. We outlined two general types of models which possessed properties consistent with the observed characteristics of immune responses regulated by Ir genes. One type of model was based upon the premise that the product of such genes are secondary receptors and amplifiers of T-cell (and possibly B-cell) function. The second set of models can be termed ‘tolerance’ models. Two cases of the latter class of hypothesis were discussed. These proposed that an animal normally synthesized and expressed a self-component which cross-reacted with the test antigen under consideration. The product of the relevant Ir gene then suppressed or masked the presence of this antigen so that the animal became a responder to it.

A number of distinct immune response genes exist and these might have their effect at a variety of points in the generation of an immune response. The models presented here serve as testable schemes for the function of those Ir genes located close to major histocompatibility loci. Definitive solution of the problem of Ir gene function must include isolation and identification of the Ir product. Techniques now exist such as lactoperoxidase-catalyzed radiiodination (Marchalonis, 1969) of cell surface proteins (Phillips & Morrison, 1970; Marchalonis et al., 1971; Baur et al., 1971) and immunological isolation of labelled products which, in principle, provide the sensitivity required to facilitate this solution. These approaches have been used successfully to isolate and partially characterize surface immunoglobulins of B-cells (Baur et al., 1971; Marchalonis et al., 1972; Kennel & Lerner, 1973) and T-cells (Marchalonis et al., 1972; Marchalonis & Cone, 1973; Moroz & Hahn, 1973). Furthermore, experimental designs based upon these principles have established binding specificity of surface immunoglobulins isolated from T-cells activated to particular antigens (Cone et al., 1972; Marchalonis & Cone, 1973; Cone & Marchalonis, 1973; Feldmann et al., 1973; Röllinghoff et al., 1973). Comparable direct binding and characterization studies are required.
to test the possibility that a non-immunoglobulin Ir gene product might function as a lymphocyte receptor for antigen.

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REFERENCES


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