Part II. Genetics of Immunoglobulins

IMMUNOGLOBULIN GENETICS IN CELLULAR IMMUNOLOGY*

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The underlying genetic controls and a definition of the number and function of immunoglobulin genes and their interactions have already been discussed by Dr. Edelman in this monograph. Therefore, I will mention only briefly the studies of Oudin and Grubb [1,2] that serologically and genetically defined the first genes controlling immunoglobulin structural determinants in rabbit and man, respectively. I shall not go through the history of subsequent studies by a large number of serologists, immunologists and geneticists, but will simply bring us to the current time when the picture has emerged of three clusters of genes, each cluster genetically unlinked to the others, determining the structure of heavy chains, κ chains and λ chains, respectively.

In no single species has a sufficient number of genes been defined in Mendelian terms to account for the variable (v) and constant (c) regions of all the immunoglobulin polypeptide chains, but by a consideration of information available in the three best studied species, human, rabbit, and mouse, we can present a rather coherent picture. We will discuss the constant region genes first because a classical genetic picture exists here.

In the human, genetic polymorphism and, therefore, the ability to localize genes in the genome to particular linkage groups and eventually to particular morphologically identifiable chromosomes exists for several IgG subclasses and one of the IgA subclasses. These genetic markers are all found to be associated with heavy chain constant regions although not all with the Fc part.[3] All these genes are very closely linked, and recombination in families has rarely if ever been observed, although recombination within the species must occur from time to time to account for different associations of alleles on particular chromosomes. In the mouse, a similar cluster of heavy chain genes that codes for the constant regions of each of the IgG classes and an IgA class has been discovered.[4] Parenthetically, I mention here that it was at the Cold Spring Harbor Symposium in 1964 that Dr. Kunkel and his coworkers and we simultaneously reported the existence of these heavy chain gene clusters in mouse and man,[5,6] respectively. In the rabbit IgG subclasses are not known, but the genes for the γ and μ constant regions that were recently studied are again very closely linked.[7]

The rabbit is the species that has provided the genetic information from which the conclusion of linkage between v and c region genes can be reached. The well-known Aa allotypes of the rabbit are v region genetic markers.[8] The reasons for talking of separate v and c genes coding for what are secreted as single polypeptide chains will also be discussed at greater length by Dr. Todd later in this monograph. Suffice it to say that the postulation of separate v and c genes can readily account for a given v region allotype synthetically

* This work was supported by United States Public Health Service grants CA-04681 and AI 08917.

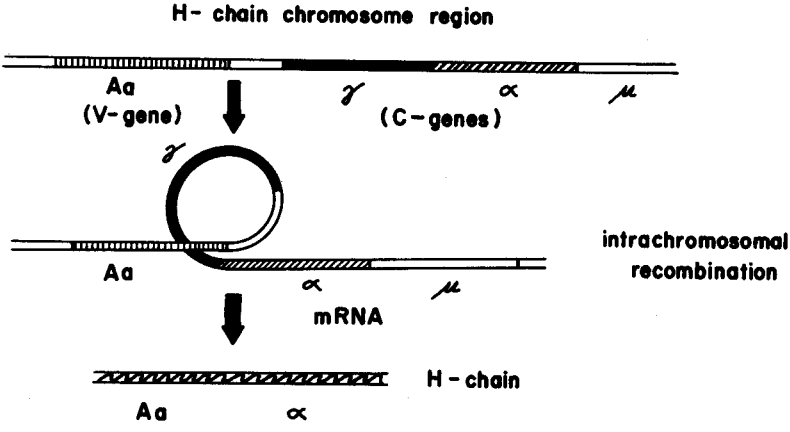

FIGURE 1.

This brief introduction to immunoglobulin genetics has discussed mainly the kind of insight into gene-protein relationships that one can obtain studying classical Mendelian character, allotypes. We know of no necessity for the existence of this type of genetic polymorphism but these allotypes do provide us with powerful markers for experimental studies of the immunoglobulins themselves and of the cells which produce these molecules.

I take this opportunity to illustrate a particularly interesting use of the mouse region allotypes as cell markers for analysis of the role of thymus-derived cells of bone marrow origin (T-cells) and non thymus-derived cells of bone marrow origin (B-cells) in immunological memory. These experiments were carried out recently in my laboratory by Dr. Graham Mitchell and in Dr. Robert Mishell's laboratory by Eva Chan. These experiments, which will be reported in detail elsewhere, take advantage of specially bred congenic strains of mice which are genetically very similar and histocompatible but which carry different immunoglobulin allotypes.[10]

It has previously been shown that B-cells give rise to antibody-producing cells, but that in a primary response, specific T-cells are required to be present in order that antibody formation may occur. In our experiments, spleen cells from mice primed weeks earlier with sheep or horse red cells were used. These spleen cell populations had both T- and B-cells, and we wished to know whether each of these cell types could exhibit immunological memory for sheep red cells. To this end, antiserum to a cell surface antigen, θ,[11] present only on the T-cells in this spleen population was used in the presence of complement to deplete the population of T-cells. Such depletion had been shown by others to abrogate the adoptive immune response that untreated spleen cell populations would give in irradiated syngeneic recipients.[12, 13] We made the same observation using congenic recipients. We found, in addition, that we could restore this response by adding back T-cells (FIGURE 2). Most interestingly, thymocytes themselves replaced the T-cells from the immunized spleen. From this we could conclude that immunological memory is carried by B-cells, but that this memory cannot be expressed in the absence of T-cells. These T-cells need not have been previously exposed to the antigen. We did

coupled and—as we shall see later in the contributions of Dr. Pink and Dr. Pernis—sequentially coupled in the same cell lineages to different heavy chain constant regions.

In the light chains, although information about Mendelian genetic markers for the v regions is almost nonexistent, the definition of an apparently discrete number of amino acid sequence subtypes suggests the existence of in the order of ten v region genes, each of which can associate with one constant region gene for the κ light chains, and another ten or so of different v region genes which can associate with one or two c region genes for λ light chains. Similar evidence for v region subtypes exists for the heavy chains and each v region subtype can be associated with each of the heavy chain constant regions. The picture thus emerges of the three gene clusters for v and c regions of the heavy chains, κ light chains and λ chains, respectively.

Sequence homologies within a cluster and general notions that such clusters can arise through evolution by gene duplication mechanisms probably explain the origin of these gene clusters, but some physiological significance must be given to the evolutionary persistence of these genes in clusters, i.e., some evolutionary advantage must be conferred by the continued close linkage of the genes rather than their being scattered throughout the genome as would tend to happen in the course of random chromosome rearrangement during evolutionary time. That these clusters do exist in all three species about which we have much knowledge of immunoglobulin genetics is not due to evolutionary oversights. The linkage is retained, I would postulate, to provide a means for orderly and perhaps sequential association of v region and c region genes in order to form complete immunoglobulin polypeptide chains.

Studies of the concomitant inheritance of Aa markers and c region markers have revealed the very close linkage between these two genetic loci. This linkage may not be as close as that between human or mouse c genes, since a single genetic recombinant between these two loci has now been observed. Since there are multiple amino acid differences between the Aa alleles, it is reasonable to consider that there may be several closely linked loci behaving as alleles in much the same way as heavy chain constant region gene clusters behave as alleles. However, whether there is one or several closely linked Aa loci, Aa is closely linked to the constant region loci for heavy chains. Thus, both v and c genes for heavy chains are in this same gene cluster.

A simple model that we proposed [9] offers a physical basis for combination of a v region gene with first one and then another c region gene on the same chromosome, but would not regularly permit association for synthesis of a messenger RNA for a complete immunoglobulin chain between v and c genes on different chromosomes. In FIGURE 1 we have shown a loop which can be of smaller or larger size but which always retains pairing between homologous, but not identical, nucleotide sequences. With one size loop, a particular v gene will be in a position where a recombinational event will join it to a particular c region gene, e.g., a μ gene. If the loop is lengthened, this same v gene can by recombination be coupled to a γ gene, for example. Since these recombinational events are intrachromosomal, this model predicts that v region and c region allotypes from the same chromosome will be found on the same immunoglobulin chains. Any allotype recombinant chains would require some, unusual, interchromosomal event. Elsewhere in his monograph, Dr. Todd discusses this subject, presenting evidence for concluding that interchromosomal v and c gene recombination in somatic cells is a rare event.

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Discussion

DR. C. J. THORBECKE: When we have done extremely similar experiments, and transferred cells after treatment with anti-theta from immune animals, we also, of course, very much abrogate the ability to transfer. But contrary to what you find we could not restore with normal thymus cells, and I think the difference might probably be in that you wait seven days after transfer before you plaque. When we plated the spleens of recipients, we did it around day 4 or 5 to bring out the big difference between secondary and primary transfers and then with normal thymus or normal spleen cells we can restore the response just about to the level that normal spleen cells would have given, but not to the immune level at all.

DR. HERZENBERG: Yes, thank you, I'm aware of your published experiments and there is this distinction. We're looking mainly at 7S plaque formers that carry the allotypes and I believe in your experiments it's mainly 19S, or it's 19S and 7S? In our experience, the peak differential response between non-immune and immune spleen cells in the adoptive transfer is at about 7 days for the 7S in particular. It's not for 19S which comes up earlier. This may be one distinction. Another distinction or a problem which may account for the differing results, which I find difficult to account for simply on the kinetic basis, is that we found recently, and Dr. Weissman at Stanford, in fact, was the first one to find it, that various anti-theta sera have other antibody activities in them as well. Some have activity against allotypes for example, and perhaps that's the most important one. We did show that the anti-theta serum which we used did not destroy plaque-forming cells but we do know, by using immunofluorescent techniques for example, that we do detect B cells with this anti-serum. It may be that more cells are being knocked out with some of the perhaps more potent sera that you have. I think the important point is that if you can restore the response, this is a positive result, while failure to restore is a negative result and we can't often reach final conclusions on the basis of negative results.

DR. WOFSY: May be it's obvious, but would you care to speculate on how you think the T cells are restoring, and why they should?

DR. HERZENBERG: You know very well that it's not obvious, but there are a couple of basic ideas. One, ascribed to Mitchison, is that the T and B cells

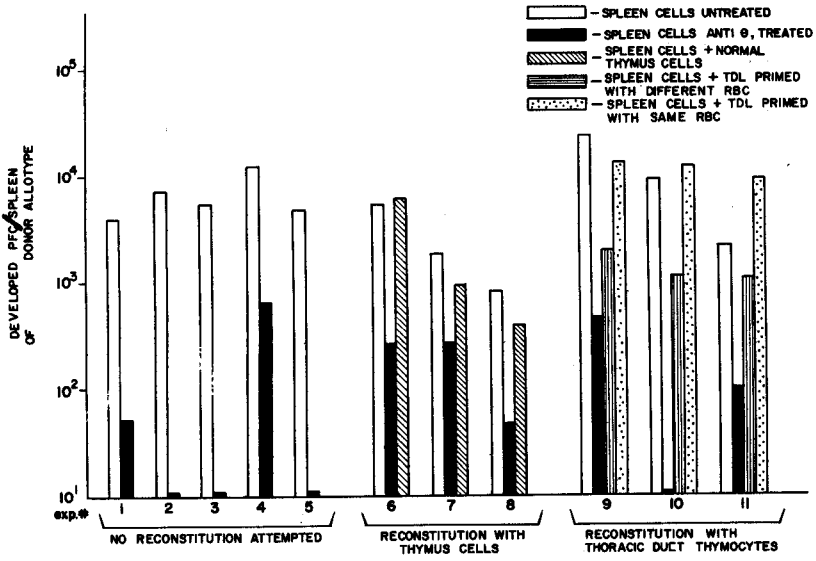

FIGURE 2. Requirement for θ antigen-bearing cells in adoptive secondary response to sheep (S) horse (H) red blood cells (RBC). In all experiments, spleen cells from C3H.SW or CWB/8 mice immunized to S or HRBC 4–16 weeks previously were treated with AKR/J anti θ^{C3H} or normal mouse serum plus guinea pig serum as complement source. One to 3 $\times$ 10^6 spleen cells were injected i.v. into 660R irradiated recipients (2–5 mice per group) of the congenic partner, with 4 $\times$ 10^8 RBC of the species used for primary immunization of spleen donors. The number of PFC developed antisera directed to the spleen donor allotype were determined seven days later. In experiments 1–5, no other cells were injected. In experiments 6–8, 2–10 $\times$ 10^7 normal thymocytes were injected with the anti θ-treated spleen cells as indicated.

In experiments 9–11, 2–2.5 $\times$ 10^6 thoracic duct lymphocytes (TDL) from animals primed four weeks earlier to H or SRBC were injected with the anti θ-treated spleen cells as indicated. The striped bars show the results when the TDL were from mice primed with the RBC other than those of the spleen cell donor; the stippled bars show results when both spleen cells and TDL donors were primed with the same RBC species.

find, however, that circulating T-cells from an animal previously exposed to sheep red cells were more effective at comparable cell doses than circulating T-cells from nonprimed animals in restoring the response of the depleted primed spleen cell population. Thus, the T-cells also have immunological memory. These experiments demonstrate that immunological memory is carried by both the B- and T-cell lines.

References

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