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A theory of allelic and isotypic exclusion for immunoglobulin genes

(heavy chain binding protein/heavy chain toxicity/DNA rearrangement)

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ABSTRACT **Heavy (H) chain binding protein (BiP), which binds to free immunoglobulin H chain of the μ and γ classes, can be demonstrated in pre-B-cells. It is proposed that the displacement of BiP from H chain by light (L) chain terminates the activity of the enzyme system, L-generase, which catalyzes DNA rearrangement at the L chain loci, generating the complete gene which may or may not be functional. This ensures allelic and isotypic exclusion for the L chain loci. It is further proposed that those cells that productively rearrange both alleles at the H chain locus are eliminated by the "H chain toxicity" effect.**

At each of the loci encoding Igs, only one (at most) of the two alleles is functional in any one lymphocyte (1, 2); this is called allelic exclusion, and it ensures that all of the antibody molecules produced by a cell have the same specificity. Furthermore, in a given lymphocyte, either κ or λ light (L) chain, but not both, can combine with heavy (H) chain to form a complete Ig molecule; this is called L chain isotypic exclusion. A simple way to achieve allelic exclusion would be to inactivate one of the homologous chromosomes, as is done with the X chromosome in female mammals (3). This is thought not to be the case; in any event, it is clear that both homologs of a chromosome cannot be completely inactivated, as would be necessary to explain L chain isotypic exclusion. For the H chain locus, it has been shown that somatic segregation (4) is not the mechanism for allelic exclusion (5). A model explaining allelic exclusion as a stochastic (chance) process of DNA rearrangement to create functional genes for Ig chains has been proposed (6), but this assumption does not, by itself, explain how the cell would stop rearrangement when it is able to synthesize a complete Ig molecule.

A note on nomenclature

We find it difficult to discuss the present problem without extending, and slightly modifying, current terminology. The recognition that Ig variable (V) and constant (C) regions must be encoded by different genetic units led to the slogan, "two genes, one polypeptide chain" (7). The folly of considering the units encoding V and C regions to be genes became fully apparent only much later when the information for a H chain was found to be encoded by four such units: *V*, *D*, *J*, and *C*. Are we to call them all genes? No, the only reasonable course seems to be to retreat back to "one gene, one polypeptide chain."‡ The H chain gene is then created by joining *V*, *D*, *J*, and *C* gene segments (or DNA segments). This is consistent with traditional genetical usage in which genes are defined by functional tests for allelism—e.g., *cis–trans* tests. We propose that this process of gene creation be called "geniture." Geniture involves DNA rearrangement, just as chromosomal inversions, deletions, and translocations are termed chromosomal rearrangements. It is, of course, possible that DNA rearrangements arise by recombination between sister chromatids. Geniture must be mediated by enzymes. We propose that these enzymes be called "generases." L-generase and H-generase mediate the geniture of L and H chain genes.

In addition to these molecular genetical neologisms, we find the need to increase the vocabulary of cellular immunology by one. In the course of B-lymphocyte differentiation, a B cell, by definition, makes complete Ig molecules. The B cell is derived from a pre-B-cell, which, by definition, makes cytoplasmic H chain only. We assume that there exists a type of cell which is already committed to the B lineage but which makes neither H nor L chain, and we propose that such a cell be called an "ur-B-cell."

Experimental basis of the theory

Unrearranged L Chain Loci. Although both alleles at the H chain locus are, as a rule, rearranged in a B lymphocyte, this is not so for the L chain loci (reviewed in ref. 8). In κ chain-producing myelomas, for instance, 50% have an unrearranged κ chain allele, and in most of these, both sets of λ chain alleles are in the germ-line configuration (6).

H Chain Binding Protein (BiP). Recently there has been found, in some cells, a protein called BiP that binds to free H chain of the μ and γ classes. BiP can be demonstrated in a murine pre-B-cell line, in some myelomas, and in hybridomas derived from them (unpublished data). It also can be seen in the polyacrylamide gel patterns of proteins from some mouse myeloma variants (9).

H Chain Toxicity. Although it is easy to isolate variants of plasma cell hybridomas that have lost expression of H chain, variants that have lost L chain expression are rare (10–12). This observation suggested that free H chain is toxic to the cell (11, 12). However, there are important exceptions to this rule. Plasma cells of people with H chain disease synthesize H chain only, as do murine pre-B-cells and hybridomas derived from them (13). In the mouse, some myeloma variants induced by chemical mutagens also produce H chain but no L chain (9). In hybridomas producing two L and two H chains, a L chain is rarely lost before a H chain, but in hybridomas producing three L and three H chains, chain loss is random (12).

The basic theory

L Chain. It is clear that L-generase activity, which promotes geniture at the L chain loci, ceases after L chain synthesis be-

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Abbreviations: H, heavy; BiP, H chain binding protein; L, light; V, variable; C, constant; ψ-H, pseudo-H; H- or L-generase, enzyme promoting gene rearrangement at the H or L chain locus, respectively.

‡ If we consider what takes place at the RNA level, we might even have to advance in reverse—i.e., one gene, many polypeptide chains.

gins. How does a cell know that a complete Ig molecule is being produced? This leads us to the first postulate of our theory.

Postulate I. *Displacement of the BiP from the H chain by the L chain terminates L-generase activity.*

There are several possibilities for the precise relationship between BiP binding and L-generase activity. Perhaps free BiP inhibits L-generase. A more interesting possibility is that the BiP–H chain complex is the L-generase. In any event, geniture at the L chain loci ceases when there is made a L chain that can combine with the preexisting H chain. In our theory, regulation of allelic exclusion for the L chain loci takes place at the genetic level rather than at the cellular level.

H Chain. Having found a putative "signal" to terminate L-generase activity, the obvious course would be to look for another signal to terminate H-generase activity. But the experimental facts discussed above seem to say (to us, at least) that there is an asymmetry or nonequivalence between H and L chains. Indeed, because unrearranged H chain alleles are rare or nonexistent in B cells, there is no reason to postulate H-generase inactivation after geniture on one homolog is achieved. The extreme form of the stochastic theory regards allelic exclusion at the H chain locus as the statistical consequence of a high error rate. Our theory accounts for allelic exclusion at this locus with the second postulate.

Postulate II. *Those cells that productively rearrange both alleles at the H chain locus are eliminated by "H chain toxicity."*

If free H chain is toxic to the cell, it seems odd for H chain synthesis to begin first (13). Perhaps the concentration of H chain in pre-B-cells is simply too low to be toxic. We know, however, that at least some of the H chain in pre-B-cells is not free—it is bound to BiP. An interesting possibility is that BiP neutralizes the toxic effect of H chain. If this is so, then the maximum amount of BiP that a cell can make must be sufficient to neutralize the amount of H chain that can be produced from one active allele but not enough to neutralize two alleles' worth of H chain. Thus, when H chain from only one homolog is being produced, BiP would prevent it from being toxic. But if a cell produced H chain from both homologs, not enough BiP would be available to prevent the toxic effect of the free H chain, and, as a consequence, the cell would be eliminated and not be seen among the population of B cells.

There are some experimental findings that are consistent with the notion that BiP is involved in neutralizing H chain toxicity. Pre-B-cell hybridomas produce large amounts of H chain; nevertheless, they grow normally and show no signs of H chain toxicity. These lines also produce large amounts of BiP, which can be precipitated along with H chain (unpublished data). BiP also was found in myeloma variants that produce H but not L chain (9). Thus, it is reasonable to assume that in all of these cells, H chain is not, in fact, free but rather is bound to BiP.

In any event, according to our theory, the regulation of allelic exclusion for the H chain locus takes place at the cellular level rather than at the genetic level. That is, those cells that do not achieve allelic exclusion at this locus are eliminated from the population of B lymphocytes.

A detailed model

The two postulates discussed above constitute the bare bones of our theory. We will now flesh them out in order to present a concrete model for the physiology of Ig gene rearrangement (Fig. 1). We imagine that an ur-B-cell makes mRNA for BiP, but that nascent BiP remains attached to the ribosomal complex until liberated by attachment to a H chain. We further imagine that the ur-B-cell is able to make a mRNA and protein chain from some part of the unrearranged H chain locus, and we call

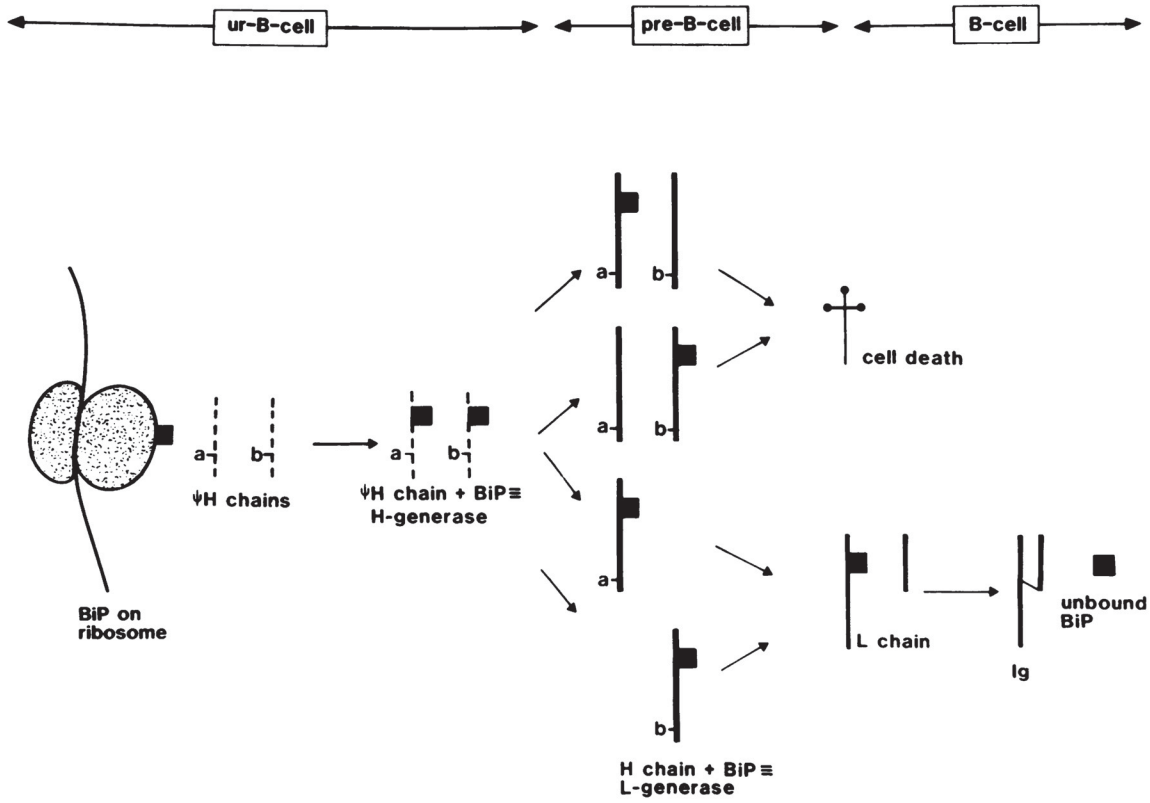

FIG. 1. Schematic illustration of the proposed model.

this pseudo-H chain (ψ-H). We note that a mRNA transcribed from an unrearranged κ chain locus has been described (14). The nascent BiP combines with ψ-H to create the H-generase, which ultimately leads to the synthesis of H chain, with the result that the ur-B-cell becomes a pre-B-cell. In this model ψ-H is part of an enzyme (H-generase) that destroys the gene (unrearranged H chain) that codes for it.

As more H chain is synthesized, it combines with nascent BiP, a process that "regulates" the amount of BiP, so that there is little or no free BiP or H chain. This type of regulation has its limits, however; when H chain is synthesized fast enough, the amount of BiP mRNA, not the freeing of nascent BiP, limits BiP production, and free toxic H chain will accumulate. As discussed above, this should happen in a cell with two productive H chain genes, and such a cell will be eliminated. The amount of BiP mRNA must, of course, vary in different types of cells; this model requires only that BiP is produced in amounts proportional to H chain. There is a precedent for this type of proportionality—the immunoglobulin molecule. Although the absolute rate of synthesis of both H and L chains differs by several orders of magnitude in small resting B lymphocytes and plasma cells, the *ratio* of H chain to L chain is essentially the same (15). We imagine that the H chain–BiP complex is the L-generase, which leads to the synthesis of L chain, with the result that the pre-B-cell becomes a B cell. The L chain displaces the BiP from the complex, and as a result, there is no generase. This displacement does not require that the initial binding of L chain to H chain be drastically stronger than that of BiP to H chain because the BiP–H chain binding is noncovalent (unpublished data), while disulfide bridges are usually formed between the L and H chains.

We will refrain from attempting to justify most of the details of the above model and will not list alternatives because we feel that it is most unlikely that any such model can be true in every detail. The point is that one can make a complete model based on our two postulates and that the model is consistent with a variety of known facts. We hope that our model will be useful in suggesting further experiments.

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