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Commentary

Fit for life in the immune system? Surrogate L chain tests H chains that test L chains

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Antibody molecules (Igs), built of heavy (H) and light (L) chains, are made by B-lymphocytes. L chains are composed of two μH chains of five Ig domains. The amino terminal domains of H and L chains are variable in amino acid sequence (V_H and V_L) particularly at the three hypervariable regions that form the antigen combining site. Assembled H-L chain pairs are stabilized by a disulfide bridge between the constant domain of the L chain and the first constant region domain of the H chain (C_{H1}). In addition, noncovalent interactions, especially between the V_H and V_L domains, are involved in H-L chain associations (1). The genes encoding Ig H and L chains are assembled by stepwise somatic rearrangements of gene segments encoding different part of the V regions (2). During B lymphocyte development in bone marrow of adult mice and humans, D_H segments first are rearranged to J_H segments, followed by V_H segments to D_HJ_H segments on the H chain loci and, finally, V_L-segment rearrangements to J_L segments on the L chain loci. Between the segments, at the joints, nontemplated N regions can be inserted, which all encode parts of the third complementary-determining regions (CDR3) of H chains, and also of L chains in humans, but not in mice. A wide variety of CDR3 amino acid sequences can be generated, as long as the nucleotide sequences remain in-frame and no stop codons were generated. It follows that these rearrangement processes also generate out-of-frame, nonfunctional H and L chain gene loci.

Different stages of pre-B cell development can be characterized by the rearrangement status of the Ig gene loci (3, 4). Even before V_H segments are rearranged to D_HJ_H segments, and well before V_L segments are rearranged to J_L segments, pre-B cells express a set of B lineage-specific genes called V_{preB} and λ_5. They encode two proteins that can assemble with each other to form an L-chain-like structure, the surrogate L chain (5–8). Although the three-dimensional structures of the V_{preB} and λ_5 proteins have not yet been determined, the nucleotide sequences of the genes encoding them have allowed predictions of their structures (Fig. 1). Thus, V_{preB} appears to have a V domain-like structure, but it lacks the last β-strand (β_7) of a typical V domain. Instead it has a carboxyl terminal end that shows no sequence homologies to any other proteins. On the other side, λ_5 appears to have a constant region—the domain with strong homologies to λL chains. Furthermore, it has, toward its amino terminal end, two functionally distinct regions. One, situated near Cλ_5, has strong homology to J regions of the λL chains, i.e., to the β_7-strand of Vλ domains. The other, i.e., the amino terminal region of the λ_5 protein, is unique in that it shows only marginal sequence homologies to Ig domain structures (5). From these sequence homologies, it has been proposed that the β_7 strand within the amino terminal portion of the λ_5 protein could provide the structure that is missing in V_{preB} to form a complete, however noncovalently assembled, Ig domain and, thus, could mediate assembly of the two proteins (Fig. 1). As reported by Minegishi, Hendershot, and Conley in this issue of the *Proceedings* (9), deletion of the

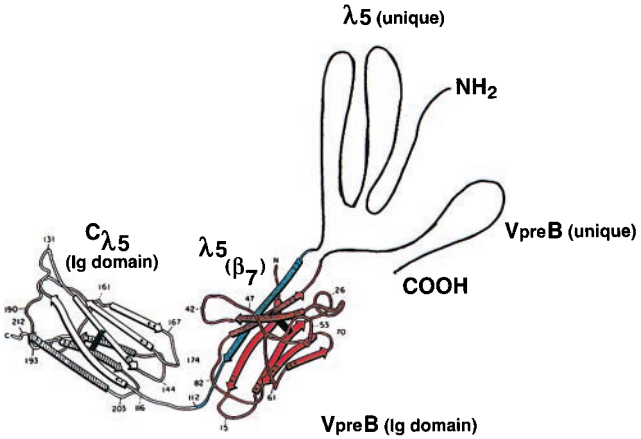

FIG. 1. Structure of associated V_{preB} and λ_5 protein as proposed by Kudo *et al.* (6), the Ig-like portions of V_{preB} (β-strands 1–6 in red) and λ_5 (Cλ_5, β-strands 1–7, amino terminal pair β_7-strand in blue) are assumed to fold as Ig domains. The figure is modified from that published in ref. 6.

β_7 strand in the amino terminal portion of the λ_5 protein completely abrogates the formation of the surrogate L chain. By measuring proper or improper folding of the protein components of the surrogate L chains and their mutants, Minegishi *et al.* conclude that complementation of the incomplete V_{preB} domain by the extra β_7-strand of λ_5 is necessary and sufficient for folding and assembly of the surrogate L chain (10–12).

Minegishi *et al.* extend their studies to show, by deletional mutagenesis of the unique amino terminal portion of λ_5 and the carboxyl terminal portion of V_{preB}, that these two parts of the proteins do not mediate assembly of the surrogate L chain, as the two deleted forms of the proteins still assemble. On the other hand, the unique amino terminal region of the λ_5 protein appears to act as an intramolecular chaperone in preventing a proper folding of the Cλ_5 domain, when it is made in the absence of its partner, V_{preB} (Fig. 2). Observations from our own laboratory support this finding, as a whole series of μH chains do not associate with λ_5 protein expressed in the absence of V_{preB} protein in the same cell (T. Seidl and F.M., unpublished work). It is tempting to speculate that the putative β-strands of the unique portion of the λ_5 protein may, in fact, disturb the proper folding of the Cλ_5 domain by assembling into the Cλ_5 fold.

Once the surrogate L chain has been assembled properly it awaits the production of a μH chain from a productively $V_HD_HJ_H$-rearranged H chain allele. Initially the μH chains bind the hsp70 chaperone BIP via their C_{H1} domains (13, 14). This prevents the C_{H1} domain from folding properly, and the μH chain is retained in the endoplasmic reticulum until BIP is replaced by a constant domain of a L chain (Hellman ☼, unpublished work as quoted by Minegishi *et al.* in ref. 9). It can

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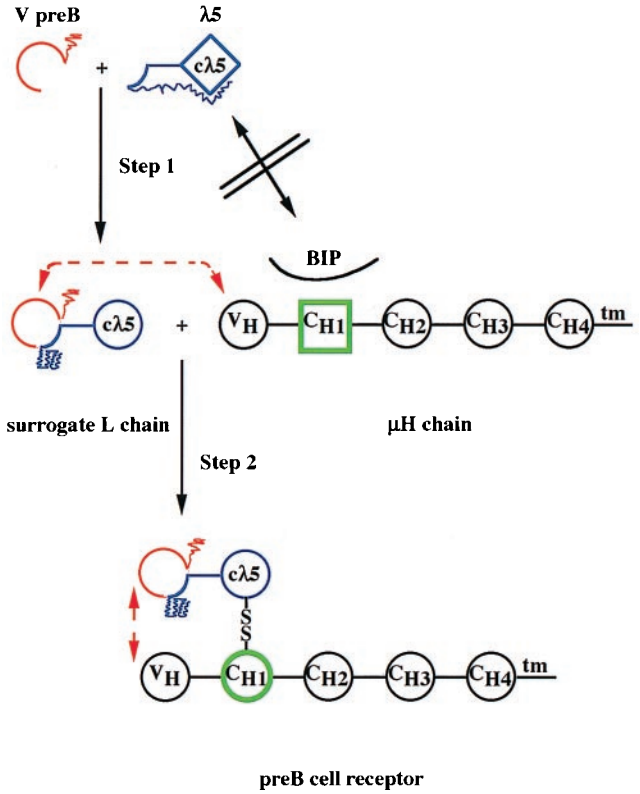

FIG. 2. Stepwise formation of the pre-B cell receptor. Isolated λ_5 protein has an improperly folded structure in which $C\lambda_5$ (◇, blue) has not yet attained an Ig-domain structure (○) and is associated with the amino terminal unique portion of λ_5, which acts as an intramolecular chaperone (〰). The β_7 strand of the amino terminal λ_5 portion is folded in an Ig-domain- like fashion (⌒). The V_{preB} protein is folded as an Ig-like domain, missing the β_7-strand with a unique, non-Ig carboxyl terminal portion (⌒, red). The isolated λ_5-protein cannot displace BIP and associate with the C_{H1}-domain of the μH chain, because neither λ_5 (◇) nor C_{H1} (□, green) are properly Ig-domain-folded. Association of V_{preB} with λ_5 induces an Ig-domain-structure in $C\lambda_5$ and displaces the intramolecular chaperone (〰). V_{preB} interacts with V_H of the μH chain, $C\lambda_5$ displaces BIP and induces an Ig-domain structure in C_{H1} (○, green), forms a disulfide bridge and thus, the pre-B cell receptor.

be expected that the properly folded $C\lambda_5$ domain in the assembled surrogate L chain can do the same to the C_{H1} domain of the μH chain, whereas free λ_5 protein, improperly folded in the absence of V_{preB}, cannot do so (Fig. 2).

However, not all μH chains, which initially are formed in pre-B cells can assemble with surrogate L chains (15, 16). In fact, to our surprise, nearly half of all newly generated μH chains were found not to be able to pair. The inability of a μH chain to pair may have more than one structural reason. Some CDR3 regions may either be too bulky or repulsive for proper interactions with surrogate L chains. Certain V_H segments carried in the H chain locus, such as the V_H81x and V_HQ52 segments, may have germ-line-encoded structural features within the V domains that do not allow proper pairing with surrogate L chains. At present, we have no good explanation why such nonfitting V-region segments would be inherited and used in the generation of μH chains.

Those μH chains that fit to surrogate L chains can form a pre-B cell receptor and display it on the cell surface. Once this has been achieved several events take place in that pre-B cell. The lymphocyte-specific components of rearrangement machinery, i.e., the genes encoding RAG-1, RAG-2, and TdT are turned off (17). That is part of the mechanism by which allelic exclusion is secured, ensuring that one of the two H chain alleles in a B lineage cell expresses a protein, i.e., one pre-B cell expresses only one type of pre-B cell receptor. Furthermore, the expression of the genes encoding the surrogate L chain genes is turned off, and remains turned off, at least, until a mature B cell has been made (5). That limits the number of surrogate L-chain molecules available in a pre-B cell for forming a pre-B cell receptor. If pairing or nonpairing of surrogate L chain with μH chains is not an all or nothing phenomenon, hence, if different avidities exist between individual μH chains and the surrogate L chain, then different concentrations of associated pre-B cell receptors may exist in and on individual pre-B cells.

The formation of pre-B cell receptors on the surface of the cells also induces them to enter cell cycle and divide several times. As the clones of pre-B cells expand they dilute the surrogate L chain molecules initially present in the first cell of the clone. If the number of surrogate L chain molecules has been reduced so much that a critical number of pre-B cell receptors is no longer available in and on a given pre-B cell

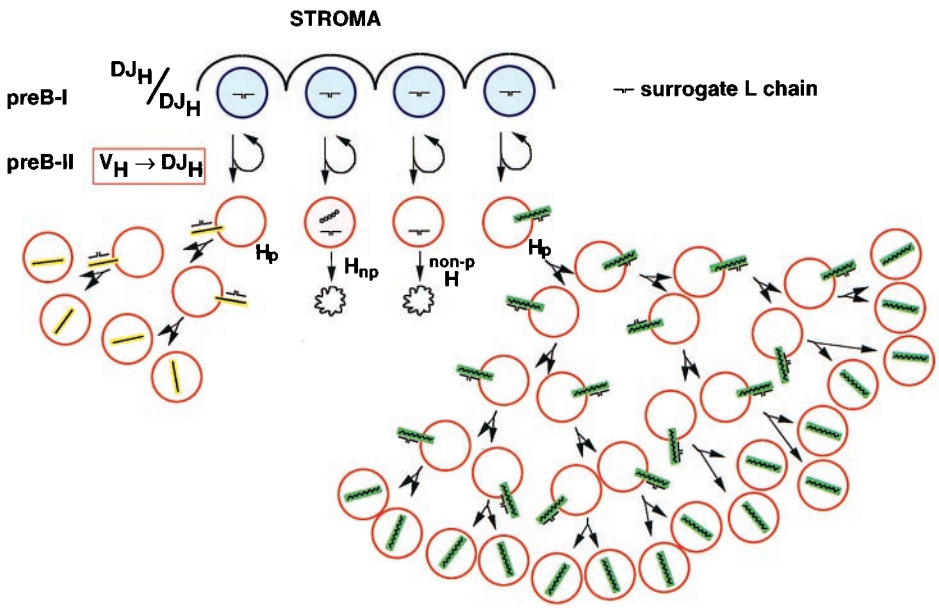

FIG. 3. Formation of the pre-B cell receptor and its effect on B cell development. DJ_H-rearranged pre-B I cells (5) in contact with bone marrow stromal cells undergo asymmetric divisions that yield one cell, which retains contact with stroma and, thus, pre-B I differentiation status. The other cell loses contact to stroma, is induced to differentiate to a pre-B II cell in rearranging a V_H to D_HJ_H segment on the H chain loci. Nonproductive (np) rearrangements leads to μH chain-negative cells that die (◌). Productive rearrangements can lead to μH chain that cannot pair with surrogate L chain (15). Cells with nonpairing μH chains cannot form a pre-B cell receptor and die. On the other hand, pairing μH chain leads to pre-B cell receptor expression on the surface, which induces pre-B II cell proliferation. The higher the avidity, the longer is the proliferative phase (high avidity = green, low avidity = yellow). In the simplest form of this model, the surrogate L chain assumes a ligand function for the μH chain to induce proliferation, hence, an external ligand binding to V_{preB} and/or V_H must not necessarily be present to induce clonal expansion of pre-B cell receptor-expressing pre-B II cells.

proliferation ceases. The higher the avidity of interaction between a μH chain and the surrogate L chain, the longer pre-B cells expressing them will expand by proliferation (Fig. 3).

Once the pre-B cell exits the cell cycle the rearrangement machinery is turned on again. However, now the κ and λL chain gene loci have become accessible for V_L to J_L rearrangements, while the H chain loci, which have not yet been $V_HD_HJ_H$-rearranged, must have previously been closed to avoid V_H to D_HJ_H rearrangements on the second allele, i.e., to maintain allelic exclusion. Once L chains are expressed from individual, productively V_LJ_L-rearranged L chain loci in clones of μH chain-expressing pre-B cells, they are tested for their capacity to pair with a given μH chain in a given cell. The properly folded C_L domain of κL or λL chains now displaces BIP on μH chain, induces proper folding of the C_{H1} domain of the μH chain and finally forms a disulfide-bonded H-L chain complex, part of the Ig molecule that then is displayed as B cell receptor for antigen on the surface. In λ_5-defective mutant mice the fitness of the V_H domains of the μH chain expressed in pre-B cells cannot be screened until L chains are expressed. V_H repertoire changes in μH chains using V_H81x and V_HQ52 segments occur in λ_5-mutant mice at the transition of a precursor to an immature surface Ig-expressing B cell, as they occur in wild-type mice at the transition from pre-B I to a pre-B II cell (Fig. 3). It remains to be seen whether surrogate L chain as stereotype for an L chain can muster all V_H domains for fitness to all V_L domains.

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