The Immune System and Bodily Defence

5. How Does the Immune System Generate a Truly Infinite Repertoire Capability?

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The authors are scientists at the National Institute of Immunology, New Delhi, and have been working on various aspects of cellular and molecular immunology for the past six years or so. In addition to teaching post-graduate immunology, their research interests and ongoing projects address the differential commitment of effector T cell pathways and signal requirements for such activation of T cells [VB], and the mechanism of antigen processing, T cell activation and tolerance, T-B cell interactions and B cell maturation [SR].

How does the immune system cut itself keys for locks it has never seen and ensure that each of its operators have only one unique key to play with?

How Do Immune Repertoire Diversities Achieve Infinity?

We have been talking last time about making a genuinely 'open-ended' repertoire for the immune system, although so far we had only managed to make a fairly large one by using a variety of tricks of gene reorganization. The only way, of course, of making a truly infinite repertoire is to mutate the VDJ or the VJ exon that codes for the variable region of each of the chains of immune receptors. Some animals, like sheep, do actually use that mechanism, and some, like chickens, use a variant of it. However, mice, the most studied creatures in immunology, and humans, who matter to us for strangely egocentric reasons, use what is, in some ways, a simpler trick. To begin with, they use a basic recombination machinery for joining V, D and J minigenes. This machinery lines up the two minigenes being joined, using sequence tags for recognition and alignment. The tag adjacent to the coding region of each minigene consists of two conserved sequences, a heptomer and a nanomer separated by either twelve or twenty three random bases (see *Figure 1*). The aligning mechanisms are such that a 7-12-9 signal tag can only align with a 7-23-9 tag and *vice versa*. Since both V and J heavy chain minigenes have the same species of tag, this system ensures that they cannot be joined by mistake to each other leaving out the D minigenes in a heavy chain.

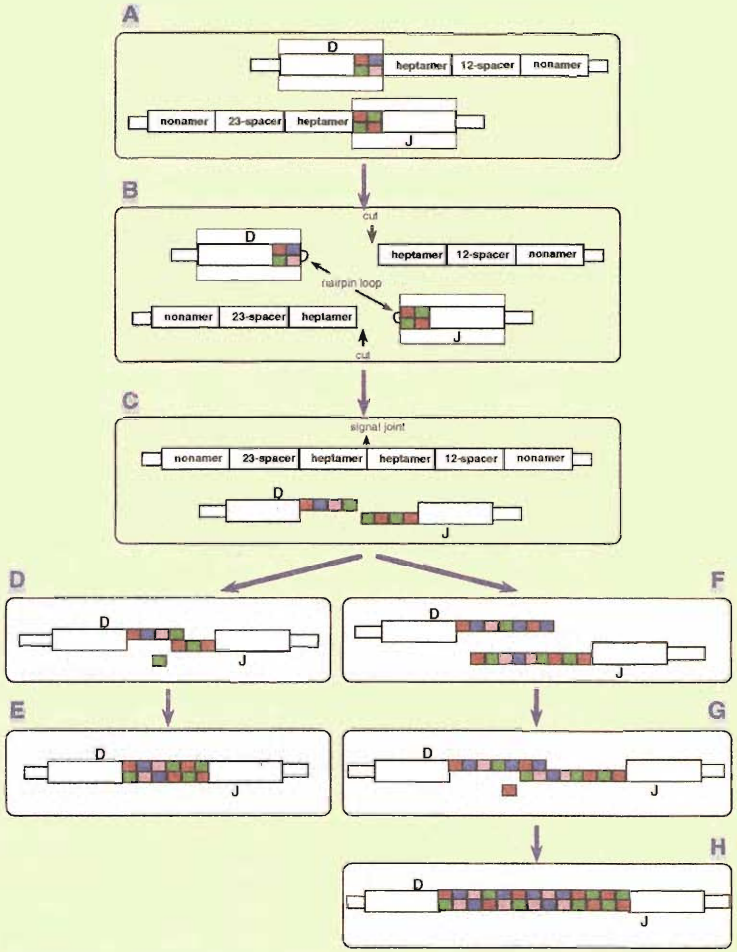

Figure 1. What happens when one of a whole bagful of D genes, for example, and one from a different basket of J genes are brought together and aligned for joining? The two minigene segments being recombined are aligned by their 7-12-9 and 7-23-9 recognition signal sequences [A]. The last two nucleotides of the coding regions of both the D and the J segments are shown in colour. After DNA cutting, a hairpin loop is formed between the two strands at the cut ends of the coding regions [B]. The loop is then 'resolved' into a single-stranded 'overhang' on the coding region ends, while the signal ends are joined to each other to form an extrachromosomal piece of DNA which is subsequently lost [C]. The overhangs are resolved in a combination of two mechanisms. One, nucleotides are removed from one or both overhanging ends by exonucleases, the D and J coding regions are aligned [D], and there is filling of the complementary nucleotides to form the completely recombined double strand [E]. The other, non-exclusive mechanism is the addition of non-templated nucleotides to the overhangs, probably by terminal deoxyribonucleotide transferase [F]. This may or may not be accompanied by base removal [G], before the strands are matched and completed [H].

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Next, the diversity-generating trick makes use of the fact that the recombination event in VDJ joining necessitates cutting one strand of the DNA double helix. This allows the cellular housekeeping machinery to use the 'nicked' strand to break the second strand as well and sticking the two open ends to each other so as to make a hairpin loop (see *Figure 1*). So now the recombining machine grips these two hairpin loops of DNA, one from each minigene to bring together for stitching, in its scaffolding frame, and cuts them open again This cutting opens the loop at a point different from the original, so now the two strands of DNA do not end at the same point; instead, one shows an 'overhang' beyond the other. This overhanging bit is very susceptible to the DNA-removing enzymes called exonucleases in the cell, which promptly hold the end in their mouth and begin chewing, and in their enthusiasm frequently chew off quite a bit more than just the overhang. This, of course, alters the sequence of DNA at the joining points in ways that were not predicted in the genome; in other words, 'non-templated' randomness has now been introduced into the VDJ exon sequence. Yet another housekeeping enzyme that can add DNA bases without having to depend on a blueprint like terminal deoxynucleotide transferase can also come in at this point and add its own two bits, changing the sequence even more, again in non-templated fashion.

Are There Differences in the Variation Patterns of B Cell Receptors and T Cell Receptors?

Thus, the true randomness in the immune repertoire is restricted to the joining areas in the VDJ or VJ regions, although as we argued last time, the VDJ combinatorial choices also provide a very substantial bulk of 'templated' but none the less extensive variability. Now, we have discussed quite early on that the target receptors on B cells and T cells recognize their targets differently. B cell receptors recognize all sorts of targets with essentially no 'spatial'

The previous articles of this series were:
1. Why do we need an immune system? January 1997.
2. How do parasites and the immune system choose their dances? February 1997.
3. How does the immune system organize itself so as to connect target recognition to expected functions? June 1997.
4. How does the immune system recognize everything under the Sun? September 1997.

constraints. However, T cells need to recognize targets as peptide fragments bound to MHC proteins on the cell surface, and these MHC proteins are not going to be infinitely variable. In fact, we have even looked at the necessity for selecting only those T cell receptors that would be able to recognize some unknown peptide fragment bound to MHC protein alleles available in the body – a process called 'positive selection'. So what are the implications of these differences in the requirements from B and T cells for the degree of variability in different parts of each receptor?

The processes of making B and T cells are quite conspicuously wasteful, and one can imagine that their utility must be very high to maintain evolutionary pressure in favour of having them!

Clearly, all parts of the B cell receptor would need a fair degree of variability, since all component regions of the receptor molecule are likely to make contact with bits of the infinitely variable world of target shapes out there. In contrast, in the T cell receptor, one would expect that the bits making contact with the MHC molecule would have less need for diversity than those regions that touch the peptide, since the peptides would be far more varied than the MHC.

What are the contributions of the VDJ minigene portions which vary in templated fashion, and the junctional regions which vary in non-templated manner, to these different areas of the T cell receptor? Interestingly, it is the peptide-contacting bits of the T cell receptor that are represented in the joining areas which show non-templated diversity. The variation in the V, D and J genes comes, naturally, from the number of different minigene alternatives available in the V, D and J clusters. Here, the T cell receptors have far fewer numbers available to them than the B cell receptors, again underlining the fact that those portions of the T cell receptor which contact MHC proteins are far less variable than those portions which connect to peptides, while on the other hand, the B cell receptors have many more minigene alternatives to choose from since they need far more all round variation. So the system finally has recourse

As soon as one of the two sites proves that it has come up with a usable set of joints for a given receptor chain, the cell will sense the presence of the chain and turn recombination off on the other chromosome.

to changing the sequence not only when diversity is needed but also in different regions within the receptors where more variation is required.

How Does Each Immune Cell have only one Receptor Despite having two Chromosomes?

While arguing for the many advantages of the clonally diverse model of immune recognition, we said it would be useful if each individual immune cell was able to recognize only one target, so that there are no unintentional mix-ups in target recognition. But if such a VDJ recombination is going to take place to make a receptor chain on each of the two chromosomes in the pair that normal people have, how does one avoid most cells coming up with two chains instead of one?

There are two ways around this. One is to rely on statistical chance. Since the VDJ recombination process is 'imprecise' because of the sequence changes being introduced at the junctions, there is only a one in three chance that the resultant joint will be 'in-frame', meaning that it will still code for amino acids instead of being complete nonsense. So the chances of both sites on a pair of chromosomes in each cell coming up with useful joints are fairly low. In fact , this is why many developing B and T cells simply die off, since they go through their attempts at recombination without ever coming up with a usable one. This makes the processes of making B and T cells quite conspicuously wasteful, and one can imagine that their utility must be very high to maintain evolutionary pressure in favour of having them!

The second mechanism to make sure that most B and T cells do not come up with more than one receptor is to play the two recombining sites off against each other. The cell sets up a race, so to say, and as soon as one of the two sites proves that it has come up with a usable set of joints for a given receptor chain, the cell will sense the presence of the chain and turn recombi-

nation off on the other chromosome. None of these mechanisms, like most things in biology, are absolute, and there are quite a few B and T cells wandering around with more than one receptor, making them potential levers for immunological confusion, especially if one of the two receptors recognises some target shape that is normal and intrinsic to the body itself! However, this is a problem that we must think about in the context of the larger issue of how the immune system weeds out such potentially harmful 'self-recognizing' cells from the developing repertoire so as to generate the 'final' repertoire that patrols the body and deals efficiently with invaders.

Suggested Reading

- **CA Janeway and P Travers. *Immunobiology : the immune system in health and disease.* Blackwell Scientific. A concise and useful textbook for serious readers in immunology.**

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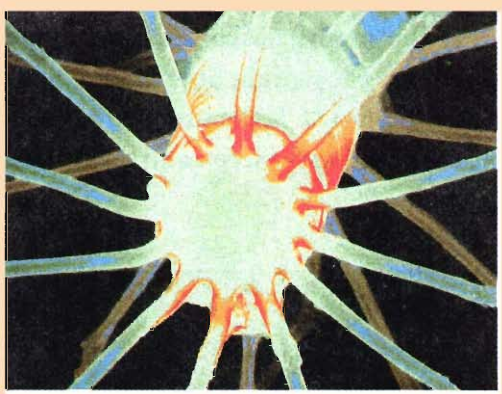

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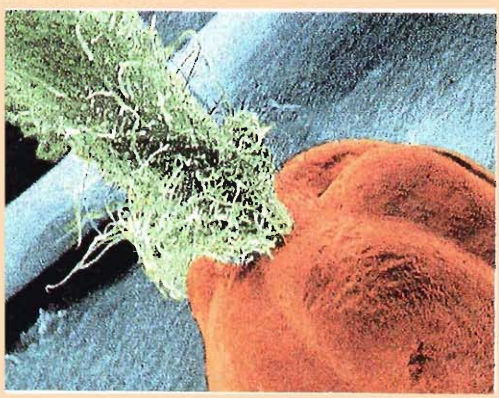

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