The Immune System and Bodily Defence

3. How Does the Immune System Organize Itself so as to Connect Target Recognition to Expected Functions?

Vineeta Bal and Satyajit Rath

How is the immune system designed to choose between making antibodies against some targets, killer cells against viral infections and helper cells for infected macrophages?

How is the Immune System Organized?

We have argued earlier that parasites can use many points of entry to begin invasion. They can enter through inhaled air like the tuberculosis bacillus; through the gut wall like the typhoid bacterium; or via skin like malarial parasites which get in through a mosquito bite. The immune system is scattered throughout the body, with its cells patrolling almost all of the extracellular space. In effect, they travel in the blood, go to the smallest capillaries, leave the blood stream there and go into the tissue space outside the blood vessels. By a matter of simple probabilities, it is in these tissue spaces that most invaders will initially land, no matter how they enter. The contents of this space drain through so-called lymphatic vessels into organs that function as 'way stations' by screening the incoming material for invaders. It is in these security stations, known as lymph nodes (such as the lumps that show up in the groin with an infected wound on the leg, or swollen glands in the throat during tonsillitis), that cells of the immune system will therefore be concentrated. Thus, after being born in the bone marrow and, in the case of so-called T cells, maturing in the thymus, cells of the immune system spend much of their life either sitting in lymph nodes, or travelling the 'policeman's beat'; the recirculating route from the blood vessels to the extracellular spaces to lymph nodes and back to the blood vessels. Immune response to invaders can therefore be

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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.

very quick, and the whole affair can be contained in the 'regional' lymph nodes rather than spreading all over.

What are the Actual Components of the Immune System?

So far, we have simply referred to 'cells of the immune system', as though they were a homogeneous population. But clearly, given the number of functions they are expected to carry out, specialization has to be the order of the day. This means that some cells would make antibodies for example, while others may activate macrophages. So who does what? To find this out, take the categories of offence-and-defence strategies that we have already talked about, and in each case look at the clonally uniform components of innate immunity and the clonally diverse players contributed by adaptive immunity.

Who Deals with Extracellular Invaders?

The first invader niche is the extracellular space. For this, phagocytic (or 'eater') cells that can engulf these invaders and kill them are needed. The clonally uniform cells which do this are the granulocytic cells, mainly the neutrophil granulocytes, and the macrophages. There are also molecular players in the clonally uniform category. Since bugs may use all sorts of tricks to become so slippery that these cells are unable to recognize them or to get a grip on them, the immune system uses various ways of 'tagging' the bug surface so that phagocytes can recognize the 'flags' and use them as handles to grab and eat the bug. Complement - a cascade of enzymatic transformations resulting in the attachment of such tags on bug surfaces, is one such player, as is C-reactive protein. Both of these recognize certain simple, common molecular shapes of bacteria that are not found in mammalian bodies, and get attached to these motifs. This can even lead to the breaking up of susceptible bacteria through the formation of 'holes' in their surfaces, and allow the phagocytes to recognize these marked bacteria and kill them.

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As usual, bugs can escape being tarred and feathered by complement and such others simply by modifying the common structures that these clonally uniform mechanisms recognize. The clonally diverse mechanisms of adaptive immunity therefore generate unique secreted proteins, - 'antibodies', - against various target structures on the bug surface. These antibodies will float around quietly in the body until they meet the target they are designed to recognize. They will then clutch this target, if possible with both their 'arms' and this binding will change the shape of their tails so as to convert them into flags marking the bugs for disposal by phagocytes. In fact, the innate and adaptive immune mechanisms interact by allowing these modified antibody tails to bind complement, providing more than one signal for phagocytes. Antibodies are immunoglobulin molecules made and secreted by plasma cells. These plasma cells are the last stages of the maturation of B cells (so called for historical reasons rather than rational ones!) responding to appropriate targets. B cells actually use an immunoglobulin as a surface molecule instead of releasing it, so that a target binding to it on the B cell surface will activate the B cell in many ways and cause their maturation into plasma cells which release the same immunoglobulin molecules. The pathways that cause optimal B cell activation involve many other cell types and need a separate discussion.

Who Deals with Intracellular Invaders?

Parasites that rush in and squeeze themselves into host cells escape being marked by antibodies or complement, and they cannot be eaten and disposed off by phagocytes on their own. Thus, the other major task of the immune system is to spot cells in which intracellular parasites are hiding. The cells of the immune system which deal with such infections [the T cells] must therefore be able to send signals to the infected cells, rather than simply releasing immunoglobulin molecules which cannot get into infected cells anyway. This means that T cells must only recognize cells as targets, not free molecules in the blood, and

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T cells can distinguish between an infected and a normal cell by just looking at their surfaces, without looking inside for the presence of the parasite itself.

release their products in a place where there are no infected cells around to be affected. So they must simultaneously recognize both a molecule unique to the parasite, as well as a marker that says that the parasite-derived target molecule is cell-associated.

If these two target recognition processes, i.e. identifying a parasite molecule and identifying a molecule that indicates the presence of a cellular target, are left independent of each other, T cells may end up effectively recognizing a free parasite-derived molecule while they are in contact with a perfectly healthy cell. They might then kill this healthy cell. Therefore, T cells must recognize parasite molecules only after they have been 'processed' or chewed up by the infected cell whose bits have then been stuck onto a normal molecule of the cell surface (*Figure 1*). These carrier molecules are coded for by genes in the Major Histocompatibility Complex (so-called, for historical rather than rational reasons!), and are therefore called MHC proteins. MHC proteins on the cell surface would thus be bound to small fragments, peptides (only eight to twenty five amino acids long), of both the host as well as parasite proteins. Thus, an infected cell can signal the presence of a parasite within it to T cells outside even if the parasite is hiding deep inside, since parasite proteins would inevitably be accessible in some quantities, at least, for degradation by the infected cell.

Though these peptides are derived from parasite molecules, their shape does not resemble the original molecules. There has to be simultaneous recognition of the MHC molecule as well. Thus, receptors that will recognise the MHC and the parasite-derived 'foreign' peptide together are needed (*Figure 1*), and the T cells bearing these receptors must then be able to either kill infected target cells, or activate them so as to help them kill their resident parasites. Incidentally, unlike many other terms in immunology, a rational reason is responsible for these cells being called T cells, because they mature in the thymus! T cells can thus distinguish between an infected and a normal cell by just looking at their surfaces, without looking inside for the presence

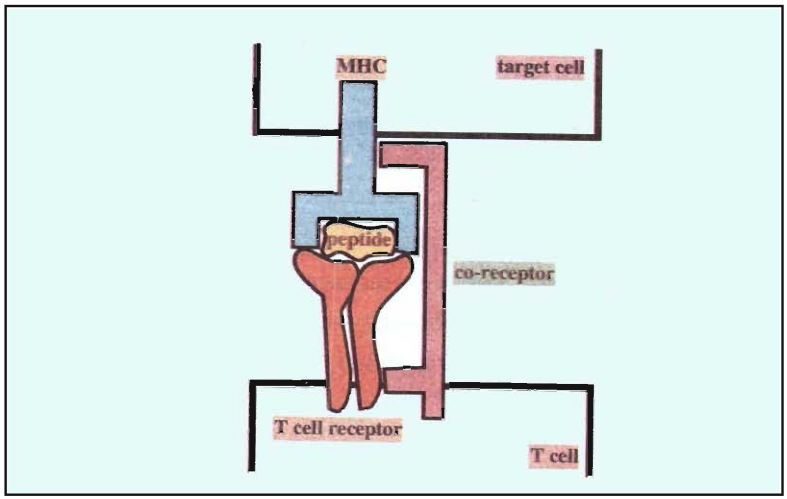

Figure 1 The T cell receptor [TCR] simultaneously recognizes both a cell surface protein [the MHC] and a peptide fragment derived from, say, the target parasite.

of the parasite itself. The T cells then make various biologically active molecules called cytokines which can transmit the appropriate signals to the infected cell.

How are Different Kinds of Intracellular Infections Identified?

We have already argued that if the parasites are sitting in the bubble organelles, or endosomes, of infected cells, these cells can, hopefully, be instructed to do something special such as turn on some enzymes or free radicals to kill the parasite. This is especially feasible because parasites will have a tendency to be taken up by the eater cells, or phagocytes, such as macrophages and contrive to sit in their endosomes without being killed. On the other hand, cytoplasmic takeover of a cell by invaders such as viruses leave very little room for hope, and the infected cell itself needs to be killed to stop the infection from spreading. So an obvious rule would be that if a T cell sees an MHC-bound peptide from a parasite protein originating in the endosomes, it should send a macrophage-activating signal saying 'kill the bug', while if it recognizes an MHC-associated peptide from a parasite protein originating in the cytoplasm, it should send a killing signal saying 'die' to the infected cell. Since these two functions are different, various subpopulations of T cells should mediate them;

The MHC class 1 molecules load peptides from cytoplasmic sources and the MHC class 2 molecules load peptides from endosomal sources.

either 'helper' T cells in the first case, or 'killer' T cells in the second. But all that T cells can see from the outside of the infected cell is a peptide-MHC complex. How can they guess where the source protein came from?

The easiest way to do this would be to have two different kinds of MHC molecules, one loading peptides from cytoplasmic sources, and the other from endosomal sources, which is what the immune system has. The first class is referred to as MHC class I, and the second as MHC class II.

How are MHC Class I Molecules Loaded with Peptides?

MHC class I molecules, like all cell proteins that have to go out onto the cell surface, are made in the tubular protein synthesis machinery of the cell - the rough endoplasmic reticulum [RER]. As soon as they are made, they are stuck into the membrane of the RER tube, and here the two proteins that together form the 'heterodimeric' MHC molecule are assembled by various molecular chaperones into a peptide-MHC complex, using any nearby peptide that happens to fit well (*Figure 2*). Where do these nearby peptides come from? Proteins that are not folded correctly while being synthesized in the cytoplasm are normally broken down by cytoplasmic factories called proteasomes. The peptides generated in this process are then pushed into the RER by a special peptide transporter protein pump in the membrane of the RER (*Figure 2*). Thus, each individual MHC class I molecule takes the peptide with which it is born out onto the cell surface. Since they come from proteasomal sources, most of these peptides are likely to be from proteins of cytoplasmic origin.

How are MHC Class II Molecules Loaded with Peptides?

MHC class II molecules, on the other hand, are born similarly, but they assemble in the RER along with a third protein called

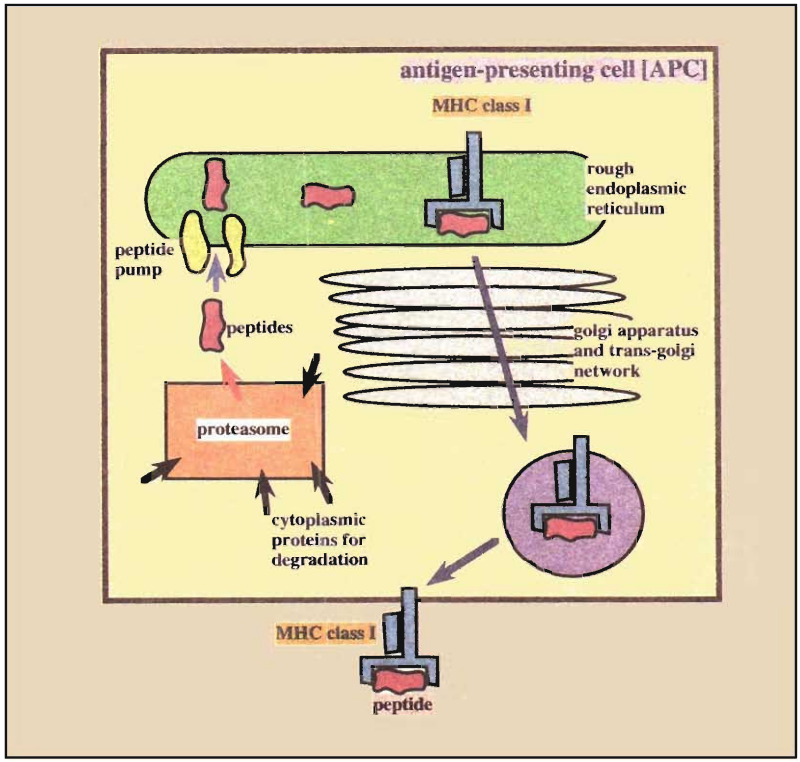

Figure 2 MHC class I proteins collect their passenger peptides in the rough endoplasmic reticulum [RER] and take them out to the cell surface. The peptides in the RER are generated in the cytoplasm by proteasomes and brought into the RER by a peptide pump.

the invariant chain, which prevents any peptides locally available in the RER from binding immediately to the newborn MHC class II molecule (*Figure 3*). They are then carried to the endosomal compartment by the invariant chain.

The endosomal compartment owes its existence to a peculiar paradox inherent in a cell bounded by a delimiting membranous wall. The components of the wall, or the plasma membrane, will be damaged with wear and tear, and must be replaced. But how can the cell pull them back in, break them down and put fresh ones in their place, all without breaching the wall in the process? The solution is an ingenious system of little transport bubbles. The cell makes a new membrane in the form of a bubble inside, takes it out to the cell surface, and patches it into the old membrane. In this process, the contents of the bubble escape into the surroundings, and this 'exocytotic' pathway is also used by cells to release all sorts of material outside. The counterflow to this path is 'endocytosis', where a little bit of the old membrane

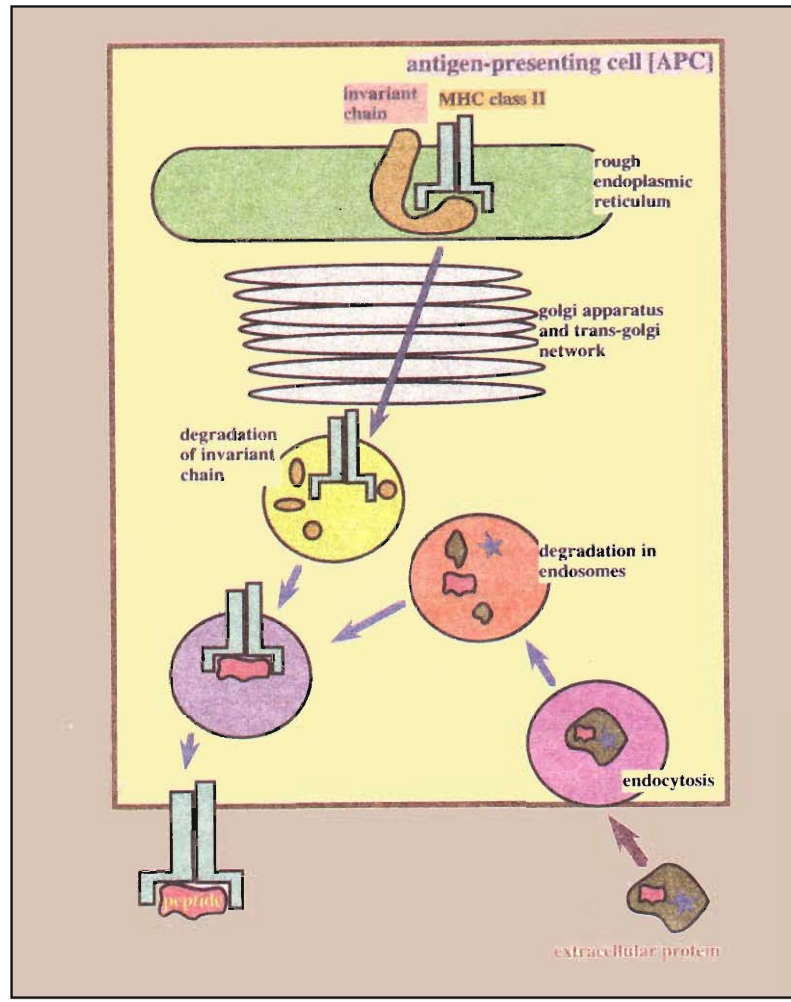

Figure 3 MHC class II molecules are protected from peptides in the RER by the invariant chain. They go to the endosomes, lose the invariant chain and take a peptide from there to the cell surface. The peptides in the endosomes are generated from membrane and extracellular proteins.

is pinched off inside the cell as a bubble, and is then carried via the endosomal compartment to waste management centres, or 'lysosomes', where it is broken down for recycling its component parts. The endocytic bubbles that are normally pinched off will carry a little outside fluid in them; a process like taking little sips of the outside fluid into the cell called 'pinocytosis' or 'cellular drinking'. Of course, the same basic pathways are exploited in taking much larger sips, or even bites of solid particulate material, or 'phagocytosis', as we have already called it.

All of these bubbles carrying both membrane and extracellular material need to be regularly broken down, and therefore the

endosomal compartment has an increasingly acidic and enzyme-rich environment. As the MHC class II molecule is brought here by the invariant chain, the chain is lost by digestion, although the MHC class II molecules are well and tightly assembled to be easily susceptible to such digestion themselves (*Figure 3*). So these free MHC class II molecules can now bind to peptides generated in the endosomes and carry them to the cell surface (these peptides are likely to be of endosomal origin). Thus, MHC class I and MHC class II molecules carry peptides of differing origins.

The recognition mechanisms of a T cell must be exceedingly sensitive in order to detect the few parasite peptide-loaded MHC molecules without losing the ability to discriminate between MHC molecules loaded with different peptides in the process.

Do MHC Molecules Bind Only to Peptides of Parasite Origin?

It is important to remember that both parasite and cellular proteins would be similarly handled by the processing mechanisms MHC molecules make no distinction between parasitic and cellular origins for the peptides they associate with. MHC molecules of either class cannot have a stable shape unless they are associated with a peptide, and even non-infected cells express copious amounts of MHC molecules. Thus most peptides on MHC molecules are of cellular rather than parasitic origin. This is the consequence of piggybacking the parasite recognition machinery onto a set of pre-existing cellular housekeeping processes that deal with normal protein turnover in the cell. Hence the majority of MHC molecules even in infected cells are likely to be carrying cellular peptides rather than parasite-derived ones. This means that the recognition mechanisms of a T cell must be exceedingly sensitive in order to detect the few parasite peptide-loaded MHC molecules without losing the ability to discriminate between MHC molecules loaded with different peptides in the process. T cells use a whole bag of tricks to increase their sensitivity without compromising their specificity.

But if T cells are so sensitive in their responses, what about the T cells that can recognize cellular peptide-MHC complexes? The T cell repertoire is randomly generated, and therefore there

is no way to stop the generation of T cells that recognize such targets. The existence of high levels of peptides of cellular origin on MHC molecules makes so-called 'autoimmunity' from these T cells a real threat, and the issue of how such 'autoreactive' T cells are to be eliminated needs separate discussion.

This scenario is further complicated by the obvious need for MHC diversity. If a given MHC molecule is to bind some peptides well, it will inevitably be unable to bind others. This means that one MHC molecule will not be able to bind all possible peptides. So MHC molecules need to be diversified in order to enable the immune system to have the chance to recognize as many parasite peptides as possible. In consequence, there have to be multiple types of MHC molecules in each of the two classes, all simultaneously being expressed on the cells of the body. But a T cell, presumably, sees only the peptide and adjacent bits of the MHC molecule with its clonally diverse T cell receptor [TCR]. So will the T cell know if it is recognizing MHC class I or MHC class II?

Why Does the Immune System Need to Select Useful T Cells?

Let us approach this class distinction from another angle. The principle of recognition of MHC-plus-peptide by T cells, coupled with this diversification of MHC molecules, causes a major problem for the development of the immune repertoire. As we have argued, the randomized generation of repertoires means that all sorts of 'shapes' of receptors will be generated. This is fine for the B cell repertoire since all that needs to be seen is parasite targets, but for T cells, no amount of invader-derived peptide recognition is likely to be useful at all unless it sees this peptide on an MHC molecule that is available in that particular individual. Randomly breeding populations, like most sexually reproducing species, would have a variety of combinations of MHC molecules of both classes, and these would be randomly reassorted during reproduction so that the child would have yet

another combination. But a randomly generated T cell repertoire will generate many receptors in each individual that can never see the MHC molecule they accidentally happen to be designed for, and these cells are never going to be of any use to that individual. So the T cell repertoire, needs to be weeded free of the T cells that recognize their target peptides on an MHC molecule that is not available in the body. This is a matter of keeping only those cells that are likely to be useful to the body (although one does not know this for sure!) and getting rid of all those that are definitely useless a process that is conveniently called 'positive selection'. At this point in the developmental decision-making process, the T cell also needs to be told which class of MHC molecule its TCR is recognizing, so that it can appropriately mature into either a helper T cell or a killer T cell.

How Does the Immune System Positively Select T Cells?

How are the elements of these processes to be controlled? Imagine that, if a T cell is going to recognize, say, peptide X on MHC A, then it is likely to bind, albeit weakly, to MHC A even when it is carrying peptide Y. So, the developing T cell should survive if and only if there is some weak interaction between its TCR and some MHC molecule in its microenvironment. So if the right MHC molecule is present, even in the absence of the right peptide, the T cell will discover that it may be useful to the body, will receive a 'rescue' signal and will survive (*Figure 4*). Otherwise it will die following a built-in programme of suicide that all developing T cells are born with.

The next problem - the two classes of MHC molecules may well distinguish between peptides of either cytoplasmic or endosomal origin, but how is a TCR to know which class of MHC it is looking at, since all it sees are the variable portions of the MHC molecule? The only practical way is to add a class-recognizing element to the TCR. So newborn T cells randomly express one of two class-recognizing molecules on its surface, one specific

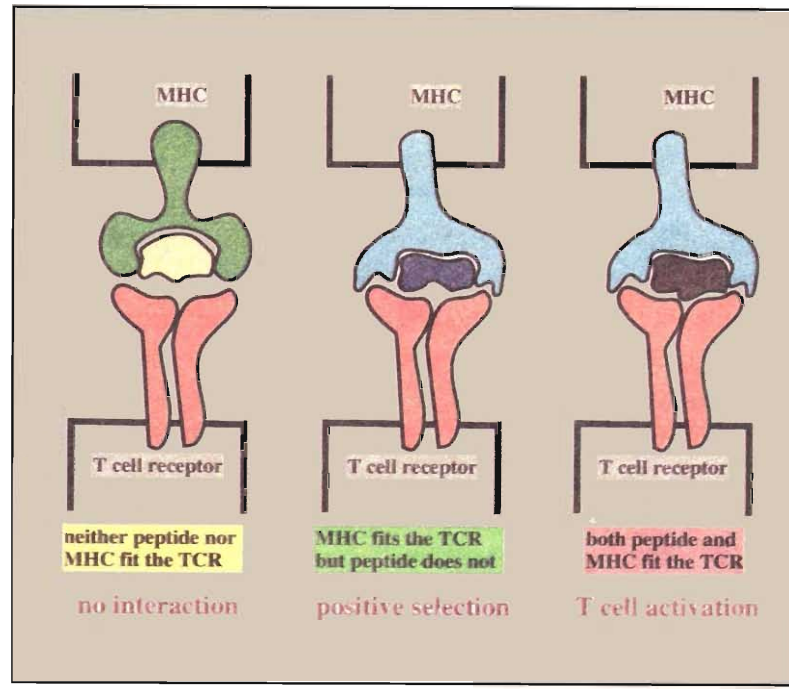

Figure 4 The goodness of fit between the peptide-MHC complex on the one hand, and the TCR on the other, decides the outcome of their interaction.

for MHC class I, called CD8, and one specific for MHC class II, called CD4. CD8-expressing T cells [CD8 T cells] would mature into killers, while CD4-expressing T cells [CD4 T cells] would grow up into helpers. Now, if a developing T cell with a TCR that binds weakly to MHC class I expresses CD8, the CD8 and the TCR will bind together to the MHC molecule, and in co-operation will provide the complete 'rescuing' signal that allows that T cell to survive and mature as a potential killer CD8 T cell. However, if this developing T cell that has an MHC class I-recognizing TCR expresses CD4 instead of CD8, its TCR will not bind to the same MHC molecule that its CD4 will recognize. In other words, there will be no MHC class-specific co-binding. This would be an inadequate signal, and the T cell would still die. Thus, MHC class recognition by the 'co-receptors', CD4 and CD8, becomes an essential component of the correct maturation of the T cell repertoire, since only those T cells that have the MHC specificity of their TCR and their co-receptors matching will survive and mature into useful functionality.

Are there Innate Immune Mechanisms Dealing with Intracellular Parasites?

All these complex arguments, creating more convoluted interactions to deal with the problems associated with careful invader identification even when they are hiding inside cells are concerned with the clonally diverse mechanisms of adaptive immunity. Does this mean that there are no cells that are clonally uniform and still capable of recognizing intracellular infections? Of course there are, and the macrophages immediately come to mind. Although facultative intracellular parasites can survive inside macrophages, evolutionary pressure in turn can help the macrophages kill some intracellular parasites with greater efficiency even in the absence of help from CD4 T cells.

However, the real teaser in this category is the so-called 'natural killer', or NK, cell. NK cells were originally identified as ones that killed tumor cells even without being previously exposed to them. However, we have argued earlier that protection against cancer is unlikely to be a major pressure in the evolution of the immune system. So are the NK cells useful in any infections? The answer is that at least in some viral infections, absence of these cells does lead to a more severe and prolonged course of infection, and NK cells do kill virus-infected cells by mechanisms similar to those used by killer CD8 T cells.

But we have been arguing that killer T cells must recognize viral peptides stuck onto MHC class I molecules on the surface of infected cells as targets, and that this is possible because killer T cells are clonally diverse. If NK cells are clonally uniform, what sort of a molecular target do they recognize?

The strategy here is to recognize some changes in the cell surface that are common to most virus infections. One easy way of doing this is to presume that cells which have had their protein factories taken over by something else are likely to be infected, and must

Suggested Reading

- **TJ Braciale and J Trowsdale. Eds. Antigen recognition. *Current Opinions in Immunology.* Vol. 5. pp. 1-55, 1993.**
- **S Rath and V Bal. How do T-lymphocytes recognise their immune targets? *Resonance.* Vol. 2.No.2. pp. 90-93, 1997.**
- **CA Janeway Jr and P Travers. *Immunobiology : the immune system in health and disease.* Blackwell Scientific publication. [A concise and useful textbook for serious readers of Immunology]**

therefore die. How does one find out from the surface if the protein factories of the cell are no longer making its own proteins? One way is to look for the levels of some marker protein. It appears that NK cells look for MHC class I molecule levels. If the levels are high, the NK cell goes away quietly. But if the level is low it will promptly label this as an 'aberrant' cell and kill it. Clearly, this suggests that NK cells must also be 'educated' to recognize what levels of MHC molecules are acceptable, and thus, selective processes must operate even on these apparently clonally uniform cells. In fact, there is every indication that they may not be completely clonally uniform, but instead may have quite some degree of diversity. However, this diversity is clearly not in the same league as that of the true adaptive immune mechanisms, where even completely synthetic 'unnatural' molecules that have never before existed can be recognized. How the truly open-ended repertoires of B and T cells are formed is thus our next concern.

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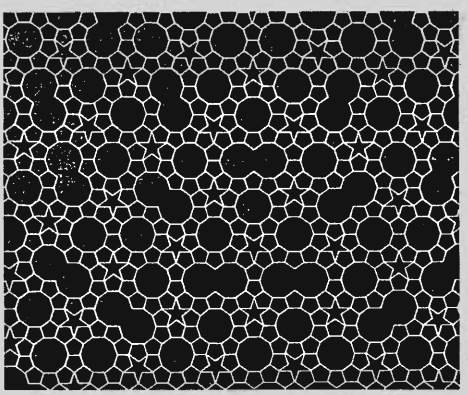

Johannes Kepler, famous for his works in astronomy, also investigated the problems associated with packing in two and three dimensions. The tiling shown here was designed by him. The pattern has reflection and rotation (by 72°) symmetries but has no translational symmetry.

From: *What Makes Nature Tick?*