Recognition by human gut γδ cells of stress inducible major histocompatibility molecules on enterocytes

By now it has become clear that IEL antigen receptor diversity is indeed limited. In the extreme, almost all γδ+ IELs of murine skin, known as DETC (dendritic epidermal T cells), express an identical antigen receptor (Vγ5, Vδ1). And functionally, DETC respond to uninfected, heat shocked murine keratinocytes. Recently, we found that in mice in which the Vγ5 gene is mutated, many of the “substitute” DETC display a γδ TCR of conserved conformation (as detected by a signatory antibody) and similar reactivity to keratinocytes.

The molecular specificity of γδ+ IELs is still unknown. Numerous studies indicated that γδ cells differ from conventional T cells in recognising antigens as diverse as non-conventional class I MHC antigens (mouse TL, Qa) and low molecular mass phosphorylated isoprenes. Now Groh et al have shown that two lines of CD4−, CD8− γδ+ IELs, explanted from cells infiltrating human intestinal tumours, recognise at least two non-conventional class I MHC gene products, MICA and MICB, expressed by intestinal epithelial tumours.

MICA is distantly related to conventional polymorphic class I MHC molecules and is thought to adopt a similar configuration, with outwardly exposed α1 and α2 domains. Unlike conventional class I MHC molecules that present peptides to cytolytic CD8+ T cells, MICA expression requires neither β2-microglobulin nor conventional antigen processing molecules. The MICA gene is preceded by a heat shock promoter, and its expression, which seems to be exclusively in patches in the gastrointestinal and thymic epithelium, may be induced by heat shock. Hence, MICA has all the hallmarks one would seek in a non-conventional MHC gene manifesting cell infection or transformation. MICB is 83% identical with MICA, particularly in the α1 and α2 domains, but has been less well studied.

Groh et al show that the γδ cell lines lysed human or mouse cells transfected with either MICA or MICB; that antibodies to MICA/B or to TCR γδ inhibited the reactivity; that the α1, α2 domains of MICA/B were necessary and sufficient for reactivity; and that recognition required neither peptide loading nor conventional class I or class II MHC. In further support of MICA/B recognition being γδ TCR mediated, the authors found that of 16 γδ clones which reacted to MICA/B, all expressed gut associated Vγ1 and Vδ1 genes, rather than the Vγ2, Vδ2 genes prevalent in peripheral blood.

Interestingly, the reactive clones showed striking diversity in the V(δ)(J) junction sequences that one would predict encode part of the antigen combining region on the TCR. Such was the diversity that it is possible that all Vγ1, Vδ1 + IELs recognise MICA/B (although the paper was unclear on the properties of clones that failed to recognise MICA/B). Based on the immunoglobulin paradigm, it is difficult to understand how high affinity recognition of an antigen can be achieved with such junctional diversity. The paper offers a clue to what may be going on.

Comment

Intraepithelial lymphocytes (IELs) are predominantly T cells and are found in the guts of vertebrates ranging from chickens through rodents to humans. To varying degrees in different species, IELs are also found in other epithelia. Murine epidermal IELs expand in areas of active hair growth, and intestinal IELs expand in response to coccidial infection of the gut. The numbers of human intestinal IELs, ordinarily a few per villus, are dramatically expanded in coeliac disease. However, neither the stimulus for, nor the consequences of IEL expansion are understood. Compounding this, IELs are invariably enriched relative to the systemic circulation in T cells expressing the γδ T cell receptor (TCR). Unexpectedly discovered in the 1980s, γδ cells are themselves an enigma. A decade ago, an hypothesis for IEL function was proposed based on the following reasoning. In the conventional immune system, intense antigen sampling in the lymph nodes allows clonal selection of relevant B cells and T cells from a massive antigen receptor repertoire generated by somatic gene rearrangement. By contrast, IELs are within epithelia, and although unlikely to be sessile, they probably encounter tens rather than tens of thousands of antigens each day. Hence for IELs to be activated, their antigen receptor diversity should be correspondingly limited. Thus, their antigens are likely to be common microbial antigens, or self antigens that are general harbingers of epithelial cell “stress”, caused by infections or cell transformation. The products of non-polymorphic major histocompatibility complex (MHC) genes, of hitherto unknown function, were proposed to be such harbingers.
The expression of MICA/B on several intestinal epithelial cell lines was shown to occur when the cells were rapidly growing or when they were heat shocked. Under these conditions, the cells were lysed by γδ+ IELs. However, non-heat shocked cells that were refractory to γδ cell recognition also expressed measurable, albeit lower, MICA/B on their surface. This is not what one expects from conventional T and B cells, which are exquisitely sensitive to low levels of their antigen. Indeed, to generate biologically safe repertoires of conventional lymphocytes, negative selection processes purge lymphocytes that recognise self antigens with high affinity. By contrast, γδ cell recognition of self antigens may be through low affinity TCR-ligand interactions, the γδ cells becoming activated by high avidity interactions, elicited by up-regulation of their antigens’ expression. In this case, many γδ cell receptors might engage a single antigen, but pathological autoreactivity toward normal tissue would be avoided by tightly limiting the expression of the antigen. Such “avidity driven” rather than “affinity driven” activation would establish γδ IELs as truly distinct. Possibly other “non-conventional” lymphocytes (for example, B1 B cells) also comply with this pattern.

Currently we remain ignorant both of MICA regulation by gut infection and of the biological relevance of the ensuing γδ response. Ironically, mice do not harbour MICA/B genes. Hence, a comparison of the responses of mice that do or (via targeted mutagenesis) do not express MICA cannot be undertaken. Although transgenic mice expressing MICA can be made, there is no evidence that mouse γδ cells will respond to MICA. Indeed, none of several antigens identified for human peripheral blood γδ stimulates murine γδ cells.

Nevertheless, it seems unlikely that murine γδ IELs will have a function entirely different from human IELs. Interestingly, γδ cell deficient, TCRδ−/− mice have so far failed to show significant deficiencies to viral, bacterial, or protozoal infections: a measurable phenotype of γδ deficiency generally only occurs in the absence of βδ T cells. If γδ IELs sit poised as sentinels of gut infection, one might have expected that γδ deficiency would be associated with increased host susceptibility. In considering this, we have hypothesised that the major contribution of γδ cells may be most significant when there is de facto, little βδ T cell function: that is, in the neonate.11 Interestingly, γδ IELs are commonly the first T cells to develop in ontogeny, making them good candidates for providing “generic” protection of the epithelial surfaces of neonates. Experiments to test this are ongoing. Provocatively, neonatal TCRδ−/− mice seem more susceptible to cryptosporidium infection than normal neonates.12

Finally, Groh et al’s findings may lead to the reconciliation of γδ cell physiology with MIC genetics. Sixteen alleles of MICA with amino acid polymorphisms in the extracellular regions have been identified.13 Another allele, with six GCT repeats in the transmembrane region, was found in 74% of 77 Japanese patients with Behçet’s disease, a multisystemic inflammatory disorder characterised by oral and genital ulcers, uveitis and skin lesions, and whose cause is unknown.14 Provocatively, Lehner and colleagues showed that γδ T cells from patients with Behçet’s disease have a specific, proliferative response to heat shock protein peptides, and that this response is not restricted by conventional MHC.15 The full tying-together of MICA genetics, heat shock protein induction, and γδ cell reactivity may be achieved in the near future, and promises to tell us much about gut associated immunology and disease.

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