Minireview

Intraepithelial γδ T cells exposed by functional genomics

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Abstract

Epithelial tissues house γδ T cells, which are important for the mucosal immune system and may be involved in controlling malignancies, infections and inflammation. Whole-genome gene-expression analysis provides a new way to study the signals required for the activation of γδ T cells, their mode of action and relationships among cells of the mucosal immune system.

T cells of the γδ subtype express on their surface a heterodimer involved in ligand recognition, the T-cell receptor (TCR), which is composed of a γ chain and δ chain distinct from, but related to, the α chain and β chain that form the TCR of αβ T cells. In peripheral blood and lymphoid organs such as spleen and lymph nodes, γδ T cells are a small proportion of T cells [1,2]. In contrast, they can constitute a large percentage of T cells within epithelia [1,2]. For example, rodent skin contains exclusively γδ intraepithelial lymphocytes (γδ IELs) [3-5] and, in most species, γδ IELs can account for as many as half of all T cells in the gut [6-8]. Activation of γδ IELs follows recognition by the TCR of ligands that remain ill-defined. Evidence that cell-surface receptors other than the TCR play a role in γδ IEL activation suggests that this process may be controlled through multiple types of receptor-ligand interactions [9-11]. The functional activities of γδ IELs have been investigated in mouse models of carcinogenesis, infection and autoimmune diseases using genetic, cellular and molecular approaches [11-15]. These studies have provided insights into a variety of functions that may be performed or controlled by γδ IELs. These functions can be grouped into the following general categories: cytolytic destruction of stressed or transformed cells; control of inflammation and developing immune responses; and modulation of epithelial cell growth. Only recently have studies begun to identify growth factors, cytokines and surface molecules involved in the recognition and effector functions of γδ IELs. Individual studies tend to remain confined to an analysis of a small number of genes or a specific cellular or molecular event. The publication of nearly complete DNA sequences for the mouse and human genomes, coupled to the availability of new gene-expression tools, now allows for more global analyses of gene expression and biological processes to be performed.

Microarrays versus SAGE: setting the stage

The number of genes involved in ligand recognition and effector functions of γδ IELs is likely to be large and, to date, these genes remain mostly unknown. It can thus be argued that, in order to understand the biology of γδ IELs, strategies capable of evaluating hundreds, if not thousands, of genes at a time can solve a major limitation of the more conventional 'hypothesis-driven' one-gene-at-a-time approach. As is often remarked, it is clear that the new gene-expression tools need careful validation when used in any particular model system. It is also obvious that traditional expertise and sound scientific judgment are more than ever required to evaluate the large amount of data generated using new gene-expression tools.

Recently, two studies in the *Proceedings of the National Academy of Sciences* [14] and in *Immunity* [16] have applied DNA microarrays and serial analysis of gene expression (SAGE) to establish the pattern of genes expressed by γδ IELs and to gain insight into their functions in epithelia. DNA arrays, used in the laboratory of Yueh-hsiu Chien [14], take advantage of available sequence information to obtain measurements of gene expression for up to tens of thousands of

genes on a single array [17]. These arrays are based on the ability of DNA or RNA labeled with a fluorescent dye, or made radioactive, to hybridize to cDNA sequences immobilized at known physical locations on a solid surface such as glass or nylon. Arrays can thus interrogate complex nucleic acid samples and provide a quantitative measure of the concentration of a specific sequence. Further analysis can be used to obtain additional information or measures such as the ratio of gene expression between different cell populations or for the same cell population subjected to different experimental conditions.

SAGE is not array-based but instead relies on compiling large cDNA libraries of expressed sequences and obtaining sequence information for short segments or tags located at the 3′ end of each cDNA [18]. This approach, used in the laboratory of Adrian Hayday [16], provides qualitative information on the identity of genes expressed. Moreover, quantitative information can be obtained from SAGE by analyzing how many times the same sequence appears. But, because SAGE relies on sequences present at the 3′ end of genes, the technique cannot discriminate the relative representation of alternatively spliced forms of RNAs that share the same 3′ end. Other disadvantages of SAGE include a need for larger amounts of good quality RNA and less sensitivity than microarrays. The modifications to the SAGE protocol reported by the Hayday laboratory [16] solve these problems to a large extent and allow application to primary cell populations available in small numbers (less than 5 million cells). One advantage of SAGE over microarrays is that it does not depend on known gene sequence information. This allows novel genes to be identified and an experimental dataset to be interrogated in the future as new genes are discovered. In contrast, a new microarray would need to be created and an experiment performed using the new array to measure the expression of a newly described gene. Nonetheless, arrays will be unparalleled tools to analyze gene expression when complete sequence information is available, including alternatively spliced forms of all genes. Tempering this enthusiasm are recent findings suggesting that it may be some time before we achieve this goal for any particular genome, and that a proposed number of approximately 30,000 genes for the human genome may require substantial upward revision [19].

Profiling γδ IELs: the gut challenge

In their study, the Chien laboratory [14] compared gene-expression profiles of purified populations of γδ IELs isolated from the gut of mice orally infected with *Yersinia pseudotuberculosis* with those of cells from the gut of control, uninfected mice. Earlier studies had shown that mice lacking γδ T cells were more sensitive than normal mice or mice lacking αβ T cells to the early dissemination of *Yersinia*, suggesting a role for γδ IELs in the control of *Yersinia* infection. This provides an interesting experimental setting in which to evaluate gene expression of γδ IELs under conditions of functional rest or activity. Fahrer *et al.* [14] also analyzed mesenteric lymph node αβ T cells bearing the TCR-coreceptor CD8 and gut epithelial cells (enterocytes), to provide cell-type controls and to compare the expression profiles of the different cell types. This study did not include an analysis of gene expression by αβ IELs, another relevant gut-resident T-cell population. Of the 6,352 genes surveyed by the microarray, some 2,100 genes were expressed by γδ IELs as well as mesenteric αβ T cells, and 800 genes were expressed by epithelial cells. Only 37 genes were found to be differentially expressed between the γδ IELs of infected and uninfected mice. The differences in expression levels were small (less than three-fold) but significant. Interestingly, none of these genes was found to be involved in an obvious way with γδ IEL activation and effector functions.

In their study, Hayday's laboratory [16] used SAGE to investigate intestinal mouse γδ IELs and αβ IELs. Hayday and colleagues generated cDNA libraries for each type of IEL and identified a total of 15,574 unique sequence tags expressed in IELs. The complete dataset is available to download from the Hayday lab's website [20]. The authors estimate that the libraries contained approximately 75% of expressed transcripts, making it difficult at this time to distinguish between rarely expressed and unexpressed genes. The Hayday study [16] finds that the majority of genes are expressed at similar levels in both IEL populations. The few genes that were overexpressed in γδ IELs compared to αβ IELs are either novel or of unknown function.

Both the Chien [14] and the Hayday [16] studies find that γδ IELs (and αβ IELs) appear to be in a state of constitutive activation compared to lymphoid $CD8^+$ αβ T cells, with high levels of expression of genes such as those encoding granzymes A and B, the apoptosis-inducing Fas ligand (FasL) and the C-C chemokine RANTES (Figure 1). The data further suggest that IELs have the potential for further activation. Despite these cells' apparent state of activation, genes encoding conventional cytokines such as the growth factor interleukin-2 (IL2), and cytokine receptors, for example the IL-2 receptor α (IL2Rα), were found to be expressed at low levels. High expression of the transcription factor JunB, which is thought to have a role in maintaining differentiated cells in a resting state, also suggests that IELs are maintained in a differentiated and resting state. This observation was confirmed by flow-cytometric analysis showing that γδ IELs expressed the early activation antigen CD69 but not the IL2Rα on the cell surface and were small, as is characteristic for resting T cells. Thus, IELs appear to be in an ill-defined state of 'restful activation' compared to lymphoid $CD8^+$ αβ T cells.

The study from Chien's laboratory [14] identifies a series of genes involved in the biosynthesis and metabolism of cholesterol and/or other lipids that are expressed by γδ IELs but

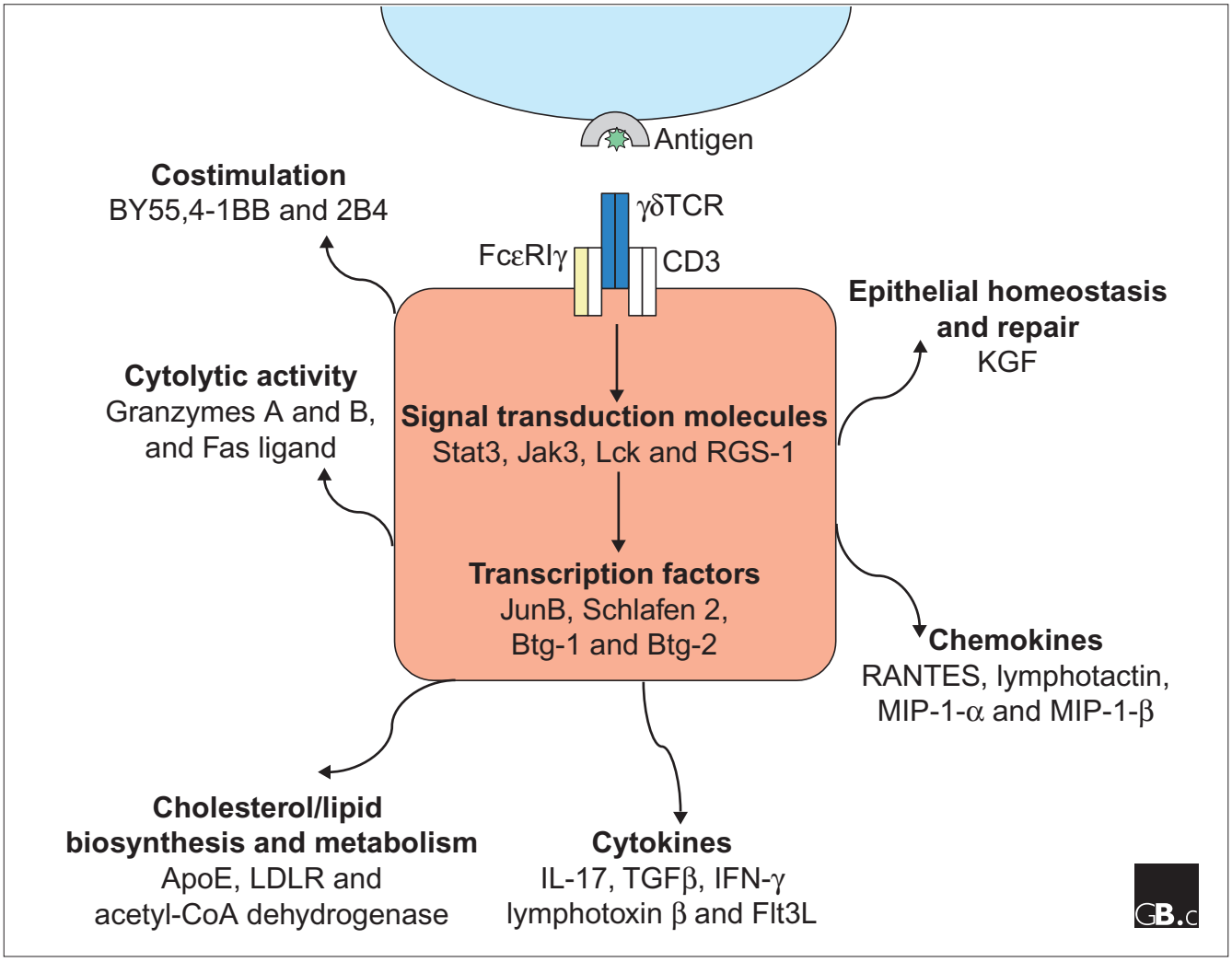

Figure 1
Summary of known and novel aspects of γδ IEL biology revealed through gene-expression studies. DNA-microarray [14] and SAGE [16] analysis provide concordant information, showing the expression of a broad range of proteins required by 'activated-yet-resting' γδ IELs for activation, function, and survival within epithelia. When a γδ IEL (shown in red) has its TCR stimulated by antigen presented on the cell surface of a stressed, transformed or infected epithelial cell (shown in blue), a signaling cascade is triggered, involving the signal transducer and activator of transcription 3 (STAT3), the tyrosine kinases Jak3 and Lck, and the regulator of G-protein signaling-1 (RGS-1). The downstream activation of various transcription factors leads to expression of proteins with diverse function. Illustrated on the figure going clockwise from top left: BY55, 4-1BB (CD137 ligand) and 2B4, co-stimulatory and accessory receptors; FcεRIγ, a receptor for the immunoglobulin E (IgE) molecule; CD3, a complex of multiple signaling chains associated with the TCR; KGF, keratinocyte growth factor; MIP-1, macrophage inflammatory protein-1; IL-17, interleukin-17; TGFβ, transforming growth factor β; INF-γ, interferon-γ; Flt3L, the ligand for the receptor tyrosine kinase Flt3; LDLR, the low-density lipoprotein receptor (LDLR). Details of other molecules mentioned in the figure are discussed in the text.

not lymphoid $CD8^+$ αβ T cells [14]. Hayday's study [16,20] found a few of these genes (for example, the gene encoding the cholesterol transport protein ApoE) expressed at low levels. At least some of these genes are not γδ IEL specific, however: they were also identified in αβ IELs. Chien and colleagues [14] also found some other genes indicative of specialized function (for example, the gene for squalene epoxidase, involved in ergosterol synthesis) expressed by both γδ IELs and enterocytes. These genes were not detected in Hayday's study [16]; this may reflect the fact that Hayday's γδ IEL cDNA library is incomplete by about 25%. A third class of related genes, such as acetyl-CoA dehydrogenase, appears to be expressed by all T cells.

The studies summarized here [14,16] distinguish γδ IELs from lymphoid $CD8^+$ αβ T cells on the basis of their gene-expression profiles or 'signatures'. It remains unclear whether or not the signatures established by these two

independent studies can be attributed to differences in T-cell lineage or to residence within different tissue environments. In this respect, future gene-expression studies of γδ IELs evaluated under different experimental conditions should provide valuable additional information. In any event, the most important lesson to be learned from the Chien [14] and Hayday [16] studies is that microarrays and SAGE, used under well-defined conditions, can provide remarkably similar data. With this 'genomic foundation' now available, the task ahead of us will be to devise ways to best exploit all this information to gain and test functional insights. This challenge announces the beginning of an exciting new era for mucosal immunologists.

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