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Vaginal microbicides may help to reduce the risk of infection with HIV or other sexually transmitted diseases. For safety evaluation of novel vaginal microbicides, vaginal irritation is generally assessed in rabbits by histopathologic analysis of the gastrouterine organs after 10 days of treatment. This evaluation requires the sacrifice of the animals. Minimally invasive approaches to measure the vaginal irritation caused by microbicides would offer a more desirable alternative. We measured the vaginal irritation caused by the spermicides nonoxonyl-9 (N-9) and benzalkonium chloride (BZK). We assessed not only the histopathological changes, but also: (a) the cytokine amounts in vaginal tissue lysates and in cotton swab samples collected from the vaginal surface, and (b) the inflammatory cells in cytobrush samples collected from the vaginal surface. As early as 6 days after beginning treatment with either N-9 or BZK, significant histopathological changes were observed. This effect was transient, being no longer detectable after 10 days. Simultaneously processed vaginal tissues contained increased amounts of CXCL8/interleukin-8, interleukin-1 β (IL-1 β), and CCL2/monocyte chemoattractant protein-1. In a second study using a minimally invasive approach, swab samples collected from the vaginal surface contained significantly elevated levels of CCL2, CXCL-8, and IL-1 β . CCL2 was significantly increased as early as 3 days after initiation of treatment with both spermicides. Increased numbers of inflammatory cells were detectable on the vaginal surface, possibly attracted by increased amounts of the inflammatory chemokines CCL2 and CXCL8. These novel findings suggest that measurement of CCL2 in particular, as well as CXCL8, IL-1 β , and inflammatory cells, on the vaginal surface may offer a new, noninvasive method to assist in developing novel vaginal microbicides.

doi:10.1016/j.cyto.2007.07.009

5 IL-25 Promotes the Initiation of Pro-allergic Type 2 Responses

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The molecular mechanisms underlying the initiation of innate and adaptive pro-allergic type 2 responses are not understood. IL-25, a member of the IL-17 cytokine family, was recently reported to be important in TH2-mediated immunity to parasitic infection. However, the cellular source and targets of IL-25 are not well understood. Here we show that mouse IL-25 is expressed by lung epithelial cells as a result of innate immune responses to allergens. Transgenic overexpression of IL-25 by these cells leads to mucus production and airway infiltration of macrophages and eosinophils whereas blockade of IL-25 conversely reduces the airway inflammation and TH2 cytokine production in an allergen-induced asthma model. In addition, IL-25, with a receptor highly expressed in TH2 than other effector T cells, promotes TH2 differentiation in an IL-4- and STAT6-dependent manner. During early T cell activation, IL-25 potentiates expression of NFATc1 and JunB transcription factors, which possibly results in increased levels of initial IL-4 production, upregulation of GATA-3 expression and enhanced TH2 differentiation. Thus, IL-25 is a critical factor regulating the initiation of innate and adaptive pro-allergic responses.

doi:10.1016/j.cyto.2007.07.010

6 Endogenous RNA Interference Influences Cytokine Gene Expression

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Helper T cell cytokine production and effector cell differentiation are influenced by endogenous RNA interference (RNAi). MicroRNA (miRNA) expression profiling and Northern blot analysis revealed that many miRNAs are rapidly downregulated following activation of naive CD4⁺ T cells, indicating that T cells 'reset' their miRNA repertoire upon activation, perhaps creating a more permissive condition for effector cell differentiation. Consistent with this hypothesis, Dicer-deficient T cells, which cannot produce miRNAs, rapidly differentiate into IFN- γ -producing Th1 effectors upon stimulation. The murine orthologue of *Caenorhabditis elegans* Eri-1 (also known as *Thex1*), an exonuclease that degrades short-interfering RNA (siRNA), is upregulated in activated T cells with kinetics that mirror the downregulation of miRNAs. Overexpression of Eri-1 decreases miRNA expression and short-interfering RNA (siRNA) function. To test whether Eri-1 regulates miRNA levels and function in T cells, we generated conditional Eri-1 'knockout' mice. T cells lacking Eri-1 expressed higher levels of miRNAs compared with wildtype controls, though activation-induced miRNA downregulation still occurs in the absence of Eri-1. As might be expected from the Th1-skewing defect of Dicer-deficient T cells, T cells lacking Eri-1 display the opposite phenotype – differentiation biased toward the Th2 lineage. Thus, endogenous RNAi appears to particularly inhibit Th1 differentiation. However, blockade of Th1 differentiation by inactivation of *Tbx21* (the gene encoding the key Th1 transcriptional regulator, T-bet), revealed that endogenous RNAi also restrains Th2 differentiation, as Dicer/*Tbx21* double-deficient T cells displayed unrestrained differentiation into IL-4-producing Th2 cells. Inactivation of a single miRNA, miR-155, also produced a mild Th2 bias, while additionally influencing cytokine production and germinal center formation by B lymphocytes. Further investigation is needed to define how specific miRNAs regulate cytokine gene expression, and how global control of miRNA homeostasis influences lymphocyte cell fate decisions and effector functions.

doi:10.1016/j.cyto.2007.07.011

7 IL-21 Induces Apoptosis of Antigen-specific CD8⁺ T Lymphocytes

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IL-21, a member of the common γ -chain family of cytokines, has pleiotropic effects on T, B and NK cells. We show that recombinant human cytokine IL-21 and the prototype common γ -chain cytokine IL-2 can both induce proliferation of anti-CD3 stimulated PBMCs. IL-21 is also able to induce STAT 3 phosphorylation, while IL-2 induces STAT 5 phosphorylation in CD3⁺ lymphocytes. IL-21R α is expressed on antigen-specific memory CD8⁺ T cells at higher levels than on unfractionated CD8⁺ T cells. Unexpectedly, we show that recombinant human IL-21-stimulated antigen-specific CD8⁺ T cells do not expand in culture like antigen-specific CD8⁺ T cells stimulated with IL-2. While recombinant human IL-21 does not stimulate antigen-specific CD8⁺ T cells to expand in culture,