CONCISE COMMUNICATIONS

Characterization of the T Cell Response to Human Rhinovirus in Children: Implications for Understanding the Immunopathology of the Common Cold

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Human rhinovirus (HRV) is a frequent respiratory pathogen, responsible for a large proportion of cases of the “common cold” and linked to acute asthma, especially in children. T cell responses to HRV and their contribution to HRV-associated pathology were investigated. T cells were obtained from tonsils removed from children at routine tonsillectomy. Proliferative and cytokine responses were measured after in vitro restimulation with purified HRV preparations of both major and minor serotypes. Most tonsils tested showed T cell proliferation, and responses to multiple serotypes in one tonsil were observed frequently. Responding T cells were CD4-positive and produced interleukin-2 and interferon-γ but no interleukin-4. Thus, children respond to HRV, a proportion of the response is serotype-cross-reactive, and a Th1 reaction predominates. In addition to contributing to protection, this response may enhance expression of virus receptor and be implicated in the immunopathology of HRV infection.

Human rhinovirus (HRV) is the most common cause of upper respiratory tract disease in humans. Although HRV seldom leads to serious illness, infection is associated with considerable loss of working time in developed countries and with other acute respiratory illness, especially exacerbations of allergic asthma, in both children and adults. Therefore, there is a need for better understanding of the immunopathology of HRV infection and of the molecular processes that link this immunopathology with other forms of pulmonary disease [1–3].

Like other picornaviruses, HRV induces serotype-specific neutralizing antibodies in both humans and experimental animals, usually recognizing only 1 of >100 known serotypes. The specificity and slowness of response, detectable in nasal secretions 2–3 weeks after infection when virus is cleared from the upper respiratory tract [4, 5], has made it difficult to devise a strategy to stimulate cross-reactive antibodies of protective value in the general population. The implication of this delay is that antibody probably plays little role in the early stages of HRV infection. Local cytokine production (e.g., interferon [IFN]) is likely to play the more dominant role during this period, although reactivation of a memory T cell response could also be a component.

Previous studies have characterized the murine T cell response to HRV [6]. HRV stimulated T cell proliferation and interleukin (IL)-2 production. In contrast to antibody, the T cell response was directed largely at internal capsid epitopes common to many serotypes. This suggested that HRV may be able to stimulate cross-reactive Th1-type responses, with implications in the natural history of the viral infection as well as in the pathogenesis of the other forms of respiratory disease. The conclusions of the murine studies were, however, limited; HRV does not produce productive infection in rodents [7].

In this study, the human T cell proliferative and cytokine responses to a variety of serotypes have been analyzed by use of human tonsil, a local nasopharyngeal mucosal lymphoid tissue where HRV infection first manifests. The cytokine spectrum demonstrated may be important not only in the immunopathology of HRV infection but also in the respiratory complications with which it has been associated.

Materials and Methods

Preparation of HRV. Methods for viral growth and purification have been described previously [6]. Virus was purified from HeLa-derived epithelial cell lysates, fractionated through a 15%–45% continuous sucrose gradient, and then detected by ELISA with HRV serotype-specific rabbit antisera. HRV-15 (major serotype) and HRV-1A or HRV-2 (two closely related members of the minor serotypes, chosen because of information available on structure) [8] were used unless otherwise indicated.

Isolation of cells from human tonsils. Tonsils obtained immediately after tonsillectomy for routine clinical indications (and in the absence of acute infection) from children between 3 and 14 years old were washed (70% ethanol), cut into 1-mm pieces, and digested (collagenase, 1 mg/mL, for 60 min at 37°C; Sigma, Poole, UK; in Hanks’ balanced salt solution [HBSS]). Digestion was...
stopped with RPMI–5% fetal calf serum (FCS), and the suspension was forced through sterile 125-μm-pore nylon mesh. Cells were washed, resuspended in 10× HBSS–isotonic Percoll, and fractionated by flotation through a discontinuous Percoll gradient (800 g, 30 min). Low-density cells (those found at the interface between 30% and 40% and between 40% and 50% Percoll) and high-density cells (50%–60% and 60%–70% interface) were collected, washed, and cultured overnight (5 × 10^6/mL in RPMI with 10% FCS, 10 mM HEPES, 2 mM l-glutamine, 50 μM 2-mercaptoethanol, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL amphotericin B [complete medium]). Media and supplements were from Life Technologies (Paisley, UK).

Enriched resting T cells were from high-density 24-h nonadherent cells (CD3, 78%; CD4, 31%; CD8, 25%) either by E-rosetting or by depletion of B cells (CD19), macrophages (CD14), and HLA-DR cells, by use of second-layer Dynabeads and rabbit anti-mouse immunoglobulin. To generate >95% CD3 T cells, low-density cells were CD3-depleted, and the remaining cells (75% CD19 B cells, and dendritic cells) were irradiated (3000 Rad, cobalt irradiation source) and used as antigen-presenting cells (APC). For CD4 and CD8 cell depletion, high-density T cells were depleted of HLA-DR, CD19, and either CD4 or CD8 cells and the required population was isolated by negative selection by use of Dynabeads (as above). Purity of the resultant populations was confirmed by flow cytometry (CD4-depleted cells: <1% CD4 cells; CD8-depleted cells: <2% CD8 cells).

**Antibodies.** Antibodies used were CD19 (BU12; gift of D. Hardie, Birmingham University, Birmingham, UK) and HLA-DR (L243), CD4 (Q4120), CD8 (UCHT14), and CD3 (UCHT1) (gifts of P. Beverley, University College London, London).

**Proliferation assays.** T cells (4 × 10^5/well) were cultured with irradiated APC (10^5/well) and 4.0 μg/mL HRV (i.e., ∼10^6 virus particles/well) or 2.5 μg/mL concanavalin A (ConA; Sigma) for 7 days in flat-bottom microtiter plates (200 μL of complete medium at 37°C, humidified 5% CO2 incubator), as based on preliminary experiments testing a range of T cell, APC, and virus concentrations and incubation times. Proliferation was measured by use of 1 μCi/well[^3H]thymidine for the final 16 h. Mean ± SD of radiolabel incorporation was calculated from triplicate wells. For some experiments, results are shown as ratio of mean incorporation in the presence and absence of virus (stimulation index [SI]).

**Cytokine assays.** Supernatants were collected and stored at −20°C. IL-4 and IFN-γ were tested by ELISA (Genzyme, Cambridge, MA). IL-2 was tested by use of a CTLL bioassay; ELISA confirmed that under these conditions, the bioassay correlated with supernatant IL-2, not with other cytokines.

**Results**

Initial experiments demonstrated an HRV-specific, dose-dependent response in T cells isolated from tonsil (data not shown). The optimal dose in preliminary experiments, 4 μg/mL, was used routinely. No response was detected when “mock” HRV from HeLa cultures lysed and purified in the same way was used (data not shown).

Figure 1A summarizes SIs when T cells plus APC from a series of 34 unselected tonsils were cultured with major (HRV-15) and minor (HRV-1A or HRV-2) forms of the virus. Of 34 tonsils tested, 27 (75%) responded (SI >2) to at least 1 serotype, with no significant difference between major and minor serotypes (average SI = 4.2 ± 2.9 and 3.9 ± 2.2), although 7 of 8 responses with SIs >9 were to the major serotype. Correlation between responses to major and minor serotypes (figure 1B) shows that in general, nonresponders responded to neither serotype (7/34), although ConA responses were normal. Overall, responses to major and minor serotypes correlated (r = .569, P < .001), although individual tonsils showing a stronger response to one virus than to another were observed.

In a proportion of randomly selected tonsils (8/34), responses to a wider panel of serotypes were tested. Individual variations in relative SI were seen, but the results shown in figure 1C are representative example: 6 of 8 tonsils responded to all 4 major HRV serotypes and 3 minor serotypes. As previously, T cells in 2 tonsils failed to respond to any HRV serotype, although responses to ConA were normal.

Infectious virus may load both class I and class II antigen-processing pathways, so in four further experiments, the phenotype of the responding T cells was examined by depletion of either CD4 or CD8 cells. CD4 cell depletion inhibited proliferation (e.g., APC + CD4 cell–depleted T cells: <4 × 10^3 cpm of[^3H]thymidine with both HRV-1A and HRV-15), whereas in the same assay, depletion of CD8 cells had little effect (e.g., APC + CD8 cell–depleted T cells: >3 × 10^4 cpm of[^3H]thymidine with HRV-1A and HRV-15; unfractionated T cells: 3.5 × 10^4 cpm of[^3H]thymidine) (SD <5%). Responder phenotyping showed increased numbers of CD4 cells at the end of the assay (data not shown), and depletion of HLA-DR-positive APC completely abolished the response to HRV (e.g., HLA-DR cell–depleted T cells + HRV-15: <2 × 10^2 cpm of[^3H]thymidine; HLA-DR cell–depleted T cells + APC + HRV-15: <1.8 × 10^4 cpm of[^3H]thymidine). Furthermore, UV-inactivated virus, unable to infect target cells, stimulated proliferation equivalent to that seen with live virus (data not shown), implying that intracellular production of viral protein was not required.

The cytokine profile of responding T cells stimulated with HRV-15 or HRV-1A is summarized in figure 2A. HRV proliferation always correlated with IL-2 and IFN-γ release, but no IL-4 (i.e., <10 pg/mL) was detected, in either proliferating or nonproliferating cultures. Figure 2B documents this further: IL-2 concentrations were maximal after 24 h, then declined rapidly; IFN-γ levels peaked at day 3 and remained high throughout the culture period.

**Discussion**

Although HRV is a common pathogen in humans, few studies have addressed cell-mediated responses to HRV. Therefore, T cell responses to HRV (both major and minor serotypes) were examined, by use of cells from tonsil, and show clearly that such responses can be demonstrated.
One potential disadvantage of using tonsillar T cells is that surgery is performed after repeated upper respiratory tract infections, raising the possibility that these tonsils are not representative. Serotype-specific proliferative responses to rhinovirus have been found in peripheral blood mononuclear cells after, but not before, priming in vivo by infection [9], and the same may apply in the tonsil. Nevertheless, the frequency of the response observed here was surprising, suggesting that most persons have responded to HRV exposure in vivo by priming a T cell antigen-specific response.

The results do not exclude HRV stimulation of primary T cell responses in vitro, or HRV acting as a superantigen, and these
Figure 2. Cytokine production (interleukin-2 [IL-2] and interferon-γ [IFN-γ]) by T cells stimulated with HRV-1A and HRV-15. A, Cytokine production in cultures with T cells, antigen-presenting cells, and either medium alone, HRV-1A, or HRV-15. Data are mean ± SE for 7 tonsils; ranges are in parentheses above bars. Comparison of cytokine release in presence or absence of virus, by use of paired Student’s t test: P < .04 (HRV-1A, IL-2), P < .02 (HRV-15, IL-2), P < .008 (HRV-1A, IFN-γ), P < .01 (HRV-15, IFN-γ). B, Time course of cytokine release. Data from 1 representative experiment of 7 summarized in A are shown.

possibilities are being explored, but preliminary work in our laboratory suggests that they are unlikely. The study, however, does clarify that in humans, as in mice, HRV is immunogenic with respect to T cells and that immunogenicity in vitro does not depend on infectivity, since inactivated virus induced similar responses. Furthermore, a proportion of these T cells may be directed at serotype–cross-reactive epitopes, since responses were either to the panel of 7 serotypes or to no virus. Future studies looking at clonal analysis will therefore be of interest.

Although the presence of a CD8 T cell response to HRV is not excluded here, and such CD8 cells will undoubtedly play a role in the elimination of infected cells, the data reported here relate primarily to CD4 cells. Responses to HRV were predominantly Th1 CD4 type, abolished by CD4 but not CD8 depletion and by removal of HLA-DR–expressing APC. Furthermore, unexpectedly for a mucosal infection, supernatants from HRV-stimulated T cells contain IL-2 and IFN-γ but no detectable IL-4. Trace undetectable IL-4 might be produced, but the balance of the cytokine profile is clearly tilted toward Th1. This is consistent with previous HRV work using peripheral blood T cells (rather than tonsil) and looking at experimental infection rather than natural history [10].

Production of Th1 cytokines in response to HRV has implications for understanding the relationship between HRV infection and host immune responses. Antigen-specific anti-HRV secretory IgA can be both neutralizing and protective against future challenge with cross-reactive virus [5]. Th1 cytokines (such as IFN-γ) may antagonize this by inhibiting the humoral responses [11] and delaying induction of neutralizing antibody [12]. On the other hand, IFN-γ may also contribute to virus clearance by increasing cellular cytotoxic responses.

Release of Th1 cytokines may not only modulate host capacity to mount effective responses to HRV but also participate in the immunopathology of HRV infection and, in particular, in linking HRV infection and acute asthma [2, 3]. In this context, IFN-γ and other Th1 cytokines can up-regulate expression
of CD54 (the major HRV receptor) and stimulate release of inflammatory mediators and intercrines from both epithelial cells [13] and macrophages, which can then act either alone or in synergy with common microorganisms. Thus, Th1 cytokines can cause strong local inflammation with little protective value but with potential for considerable local damage to host tissues.

Thus, these studies contribute to the hypothesis that HRV-associated pathology may not be due directly to viral cytopathic effects but rather to inappropriate stimulation of local inflammation. This is consistent with clinical observations that exogenous IFN-γ enhances symptoms of HRV infection, with no protective role [14]; with restriction of viral replication to few epithelial cells [15]; with association of HRV infection and lymphocytic or granulocytic infiltrates in the bronchial submucosa [3]; and with infection of epithelial cells stimulating cytokine production [13]. Under normal conditions, this will be self-limiting, resulting in mild symptoms of the common cold, but where the airways have been sensitized (e.g., in chronic allergy), HRV-induced immunopathologic responses may trigger serious “bystander” secondary symptoms, as in acute asthma. Therefore, immunologic intervention in HRV infection aiming to alleviate complications should perhaps be targeted toward switching the cytokine balance of the host response.

Acknowledgments

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References