

VICIA VILLOSA AGGLUTININ SEPARATES FRESHLY
ISOLATED PEYER'S PATCH T CELLS INTO INTERLEUKIN 5-
OR INTERLEUKIN 2-PRODUCING SUBSETS

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Lymphokines secreted by Th cells can influence the Ig isotype produced by B cells. Thus, IL-4 increases IgE and IgG1, whereas IFN- γ increases IgG2a responses of LPS-activated murine B cells (1-3). Furthermore, we and others have noted that IL-5 can act as an IgA differentiation factor (4-7).

Murine Th cell clones can be divided in two subsets, Th1 and Th2, based on the pattern of lymphokines they produce. Th1 cells secrete IL-2 and IFN- γ , but not IL-4 and IL-5 (8). Th2 cells secrete IL-4 and IL-5, but not IL-2 or IFN- γ (8). Both subsets have been identified among continuous lines and clones of L3T4-bearing T cells (8). However, Th2 cells have not been readily demonstrated among freshly isolated T cells (9). Although the mAb MRC OX-22 appears to distinguish IL-2 from non-IL-2-producing T cells in the rat (10), it has not been possible thus far to distinguish murine Th1 and Th2 cells based on cell surface properties.

Peyer's Patches (PP) in the intestinal mucosa are a major source of precursor IgA-producing cells (11). Furthermore, PP T cells are reported to have a specialized role in regulating the production of IgA by PP B cells (12-15). Given the activity of IL-5 as an IgA differentiation factor, the presence of IL-5-producing T cells in PP could have important implications for the development and regulation of mucosal IgA B cells. We demonstrate herein that freshly isolated PP contain a subset of L3T4 T cells that, when stimulated, secrete IL-5 but not IL-2. Moreover, these Th2-like cells are readily separated from IL-2-secreting PP T cells by their differential adherence to *Vicia villosa* agglutinin.

Materials and Methods

Mice. BALB/c mice (8-12 wk) were bred in our laboratories from breeding stock originally obtained from the Strong Research Foundation, San Diego, CA.

Antibodies. TRFK 5 and TRFK 4, rat IgG1 mAbs against IL-5 (16), were a generous gift of Robert Coffman, DNAX Corp. (Palo Alto, CA). Anti-IL-4 mAb 11B11 (17), mAb J11d.2 (18), anti-Lyt-2.2 mAb AD4.5 (19), anti-Thy-1.2 mAb HO13.4 (20), anti-H-2^d mAb 18-16-8S (21), and anti-L3T4 mAb GK 1.5 (22) were obtained from the American Type Culture Collection (Rockville, MD).

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Lymphokines. Highly purified IL-5 and IL-4 were provided by D. T. McKenzie, University of California at San Diego (23). 1 U of IL-5 activity is defined as yielding 50% of the maximal proliferative response in the BCL-1 assay (23). IL-5 used for generating standard curves had a specific activity of 16.5 U/ng. Recombinant human IL-1 β (Lot no. 10735) was obtained from Genzyme Corp. (Boston, MA). Recombinant human IL-2 (Lot no. LP-377) was a gift from Cetus Corp., Emeryville, CA.

Cell Preparations. PP lymphocytes were prepared as described before (4). To obtain L3T4⁺ T cells, PP cell suspensions were treated with AD4.5, J11d.2, and 28-16-8S mAbs and goat anti-mouse Ig (Southern Biotechnology Associates, Inc., Birmingham, AL) plus Low-tox rabbit complement (Accurate Chemical Co., Westbury, NY). Spleen B cells were prepared by treatment of spleen cell suspensions with a cocktail of HO13.4 and AD4.5 mAbs and Low-tox rabbit complement.

Vicia villosa Agglutinin (VVA) Panning. Panning of T cells on VVA was performed as described by others (24). Briefly, VVA (E. Y. Laboratories Inc., San Mateo, CA) was added to petri dishes at a concentration of 0.001%. The plates were then incubated for 12 h at 4°C, washed three times with PBS, pH 7.2, and incubated with 2% FCS in PBS for 15 min at 25°C. PP T cells were added at 2 × 10⁶ cells/ml in 5% FCS/PBS for 70 min at 4°C, after which nonadherent cells were removed and the plates were washed three times with PBS. Adherent cells were eluted by incubation with *N*-acetyl-D-galactosamine (1 mg/ml) in PBS at 37°C for 20 min. The plated population was 85% L3T4 positive by flow cytometry. VVA adherent cells were 10% and VVA nonadherent T cells were 90% of the total plated T cells. The recovery of L3T4⁺ cells was the same in both populations. Recovered cells were >90% viable by trypan blue exclusion.

Cell Culture. 10⁵ PP T cells were cultured in flat-bottomed, 96-well plates (Costar, Cambridge, MA) in complete medium (RPMI 1640 containing glutamine [2.4 mM], 2-ME [5 × 10⁵ M], penicillin [40 U/ml], streptomycin [40 μg/ml], 12% nutritional cocktail, and 10% FCS [Irvine Scientific, Santa Ana, CA]) in total volume of 195 μl. Lymphokines and mitogens were added at the initiation of culture in a 5 μl volume. For each addition, duplicate cultures were plated. After 15–20 h of culture, cell supernatants were harvested and assayed for IL-5, IL-2 and IL-4 activity.

Spleen B cells were cultured as described before (4) and stimulated with bacterial LPS (12.5 μg/ml: *Escherichia coli* 0127:B8; Sigma Chemical Co., St. Louis, MO) at the initiation of culture. 1 d later, titrated doses of cell supernatants from VVA adherent and VVA nonadherent T cell cultures or purified lymphokines were added. All cultures were done in triplicate. On day 7, cultures were assayed for isotype-specific antibody by ELISA as described before (4).

IL-5 ELISA Assay. The IL-5 ELISA was performed as described by others (16). Briefly, microtiter plates were coated with 1 μg/ml anti-IL-5 coating antibody in PBS and incubated at 37°C for 2 h, after which wells were blocked with 1% BSA/PBS. Test samples and dilutions of standards were added in RPMI 1640 containing 10% FCS and incubated for 2 h at 20°C. After washing the plates, 50 μl of a second, biotinylated, anti-IL-5 antibody was added for 1 h. Plates were then incubated with streptavidin-horseradish peroxidase conjugate for 1 h at room temperature. Wells were developed with 100 μl of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) in the presence of H₂O₂ and the optical density was measured at 405 nm using a Titertek Multiskan ELISA reader (Flow Laboratories, Inc., McLean, VA). The assay was sensitive to 100 pg/ml.

NK Assay. IL-2 secretion was measured using the NK proliferation assay described before (25). IL-4 activity was inhibited by the addition of anti-IL-4 mAb 11B11. In repeated experiments, IL-2 secretion was maximal between 15 and 20 h of culture.

Results

VVA Nonadherent T Cells, but not VVA Adherent T Cells Secrete IL-2. We first tested the ability of VVA nonadherent or VVA adherent Peyer's Patch L3T4 T cells to secrete IL-2. Freshly isolated T cells were stimulated for 15–20 h in culture with Con A (2 μg/ml) or Con A plus IL-1 (10 U/ml). As shown in Fig. 1, the VVA nonad-

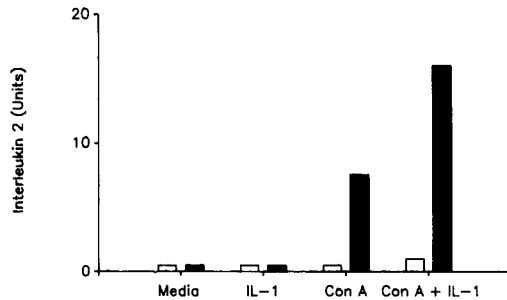


FIGURE 1. VVA nonadherent T cells, but not VVA adherent T cells secrete IL-2. Representative experiment in which 10^5 freshly isolated VVA adherent (□) or VVA nonadherent (■) Th cells from PP were stimulated with recombinant human IL-1 β (10 U/ml), Con A (2 μ g/ml), or both. Supernatants were harvested 20 h later and assayed for IL-2 activity as described in Materials and Methods.

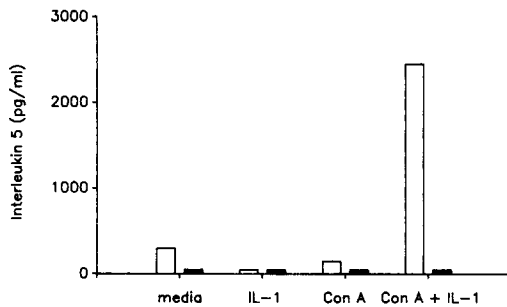


FIGURE 2. VVA adherent T cells but not VVA nonadherent T cells secrete IL-5. Representative experiment in which 10^5 freshly isolated VVA adherent (□) or VVA nonadherent (■) Th cells from PP were stimulated with recombinant human IL-1 β (10 U/ml), Con A (2 μ g/ml), or both. Supernatants were harvested 20 h later and assayed for IL-5 by ELISA.

herent cells, but not the VVA adherent cells, secreted IL-2 in response to Con A or Con A plus IL-1. Unfractionated L3T4 PP T cells also produced IL-2 after stimulation with Con A or Con A plus IL-1. The same pattern of lymphokine secretion was noted when these cells were stimulated with PHA (5 μ g/ml) or PWM (2 μ g/ml) and IL-1 (data not shown).

VVA Adherent T Cells, but not VVA Nonadherent T Cells Secrete IL-5. As shown in Fig. 2, VVA adherent T cells, produced IL-5 after stimulation with Con A plus IL-1. VVA non-adherent cells (Fig. 2) and unfractionated L3T4 PP T cells (not shown) did not produce IL-5 in response to the same stimuli. VVA adherent cells did not produce IL-5 or IL-2 in response to Con A or IL-1 alone (Figs. 1 and 2). In controls, VVA nonadherent T cells incubated with *N*-acetyl-D-galactosamine before use and reconstituted populations that consisted of 90% VVA nonadherent and 10% VVA adherent T cells, also produced IL-2, and not IL-5.

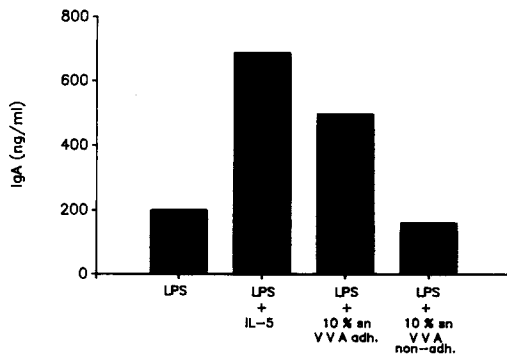


FIGURE 3. Supernatants from VVA adherent cells stimulate IgA secretion. Spleen B cells (10^5 /culture) were stimulated with LPS (12.5 μ g/ml). Highly purified IL-5 (6 ng/ml) or supernatants from Con A (2 μ g/ml) and IL-1 (10 U/ml)-stimulated VVA adherent T cells (IL-5 concentration measured by ELISA was 2.8 ng/ml) or VVA nonadherent T cells were added on day 1 of culture. Supernatants were harvested on day 7 and assayed for isotype specific antibody by ELISA.

Supernatants from VVA Adherent PP T Cells Stimulate IgA Secretion. IL-5-containing supernatants from Con A-stimulated PP T cell lines or clones were shown to enhance IgA production by LPS-activated spleen B cells (4). Thus, supernatants from VVA adherent and nonadherent PP T cell cultures were tested for their ability to enhance IgA secretion by LPS stimulated splenic B cells. As shown in Fig. 3, supernatants from Con A and IL-1 stimulated VVA adherent, but not VVA nonadherent, PP T cells enhanced IgA secretion in parallel to purified IL-5. The same supernatants did not increase IgG1 secretion (not shown).

Discussion

These studies demonstrate that freshly isolated PP contain a population of T cells that can be stimulated to IL-5 production. The IL-5-producing T cell population was revealed only when freshly isolated L3T4 PP T cells were differentially fractionated on VVA. Thus, VVA adherent PP T cells secreted IL-5 but not IL-2, whereas VVA nonadherent T cells produced IL-2, but not IL-5. In contrast, L3T4-enriched PP T cells, that were not separated on VVA, produced IL-2 and not IL-5 after stimulation with mitogens. Although spleen T cells contained only 1-2% VVA adherent cells, those cells also could be stimulated to secrete IL-5 (not shown).

Th2 cells that produce IL-5 and IL-4, but not IL-2 and IFN- γ , were described initially based on studies using long-term T cell lines and clones (8). However, it has been difficult to detect the analogous T cell subset among populations of freshly isolated T cells, despite the use of a variety of stimulation protocols. Recently, the development of IL-5-producing T cells was reported to require the repetitive stimulation in culture of antigen-primed T cells (9, 26). Those studies suggested that Th2 cells are not present or are present normally only as a minor population in lymphoid organs (9). In striking contrast, our demonstration of IL-5-producing cells in freshly isolated PP clearly indicates that such cells are present in vivo. In this regard, IL-5-producing T cells may be particularly important in the development of IgA responses in mucosal sites.

Three further points warrant comment. First, we note that T cell lines developed from freshly isolated VVA adherent PP cells preferentially produce IL-5, whereas those developed from VVA nonadherent cells secrete IL-2 (Schoenbeck, S., and M. Kagnoff, manuscript in preparation). Second, we point out that the freshly isolated VVA adherent PP T cells appear to differ in some respects from the IL-5- and IL-4-secreting T cells described among T cell clones. Thus, IL-4 was not detected in the supernatants of the VVA adherent cells, either by bioassay or by an increase in IgG1 secretion by LPS-stimulated B cells. This suggests that the coordinate production of IL-4 and IL-5 by Th2 cells from T cell clones may not be paralleled in vivo. It is tempting to speculate that an analogous dichotomy may exist in the production in vivo of IL-2 or IFN- γ . Finally, we note that populations of VVA adherent PP T cells have been proposed to regulate IgA secretion and the development of "oral tolerance" by functioning as contrasuppressor cells (24, 27). The data herein suggest that "contrasuppressor activity" in such experimental systems may, in part, be mediated by IL-5 produced by PP Th cells.

Summary

Murine L3T4 T cells freshly isolated from Peyer's Patch were fractionated based on differential adherence to *Vicia villosa* agglutinin (VVA). VVA adherent cells secreted

IL-5, but not IL-2, after stimulation with Con A and IL-1. In striking contrast, VVA nonadherent PP L3T4 T cells secreted IL-2, but not IL-5, under the same conditions. In addition, supernatants from VVA adherent, but not from VVA nonadherent cell cultures, enhanced IgA secretion by LPS-stimulated splenic B cells to the same extent as purified IL-5. Thus, IL-5-producing T cells are present in PP in situ and may play an important role in the development of mucosal immunity. Further, differential adherence to VVA can be used to separate T cell populations that preferentially secrete IL-5 or IL-2.

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