

MURINE HEPATIC ACCESSORY CELLS SUPPORT THE
PROLIFERATION OF Th1 BUT NOT Th2
HELPER T LYMPHOCYTE CLONES

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The murine liver contains cells having accessory function *in vitro* in both primary and secondary immune responses. These hepatic APC can stimulate the proliferation of allogeneic T cells (1), support T lymphocyte activation by mitogens (2), and present soluble antigens to primed T cells in an antigen-specific and genetically restricted manner (3). However, although the liver is the major site for the clearance and degradation of foreign antigens from the portal circulation, antibody responses to orally or intraperitoneally administered antigens are uncommon. Indeed, several investigators have suggested that sequestration of particulate or soluble antigens in the liver may result in tolerance to those antigens (4, 5).

The apparent discrepancy between these *in vivo* and *in vitro* observations may be explained by an inability of hepatic APC to stimulate specific subsets of T lymphocytes. Recent studies have suggested a subdivision of CD4⁺ helper T lymphocytes (HTL) into at least two categories on the basis of secreted lymphokines. According to the classification scheme first proposed by Mosmann (6), Th1 cells produce IL-2 and IFN- γ ; whereas Th2 cells produce IL-4 and IL-5. Cells of both subsets can produce IL-3, GM-CSF, and TNF. Functional differences between these HTL subsets have also been reported: Th1 cells mediate delayed-type hypersensitivity reactions and suppress specific antibody responses; in contrast, Th2 cells provide help for specific antibody production, particularly of the IgG1 and IgE isotypes. Using a panel of murine Th1 and Th2 clones as responders, we report here that freshly isolated hepatic nonparenchymal cells (NPC) from normal mice support the proliferation of Th1 but not Th2 HTL clones.

Materials and Methods

Animals, Reagents, and Media. Female C3H/HeN and B10.A mice (8–12-wk-old) were obtained from colonies from The Jackson Laboratory, Bar Harbor, ME. The mice were screened routinely for pathogens and housed in a barrier facility with sterile caging, bedding, water, and food. OVA, collagenase IV, and metrizamide were purchased from Sigma Chemical Co.,

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St. Louis, MO. DME supplemented with 10 mM 3 (*N*-morpholino) propane sulfonic acid, 0.1 mM nonessential amino acids, 5×10^{-5} M 2-ME, 5% FCS, 50 U penicillin, and 50 μ g of streptomycin per 100 ml of medium was used, except as noted in the text, for all cell preparations and cultures.

Antibodies and Recombinant Lymphokines. Hybridoma cell lines 11B11 (anti-IL-4) and S4B6 (anti-IL-2) were kindly provided by Dr. W. Paul (National Institutes of Health, Bethesda, MD) and Dr. T. Mosmann (DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, CA), respectively. Murine rIL-1, rIL-4, rIL-6, and rIL-7 were generous gifts of Immunex (Seattle, WA). Human rIL-2 was the generous gift of Cetus Corp. (Emeryville, CA).

Hepatic Nonparenchymal Cell (NPC) and Spleen Cell Isolation. Hepatic NPC and spleen cells were isolated as described previously (1). After excision of the spleen, the liver was perfused with collagenase IV via the portal vein. Nonparenchymal cells were separated from hepatocytes and debris by metrizamide density centrifugation and, after washing, resuspended in complete media. Total recovery ranged from 0.6 to 1.0×10^7 NPC per liver. 35–40% of the NPC were adherent to plastic, phagocytic, and nonspecific esterase positive. In selected experiments, hepatic NPC and splenic cells were adhered to 24-well plate plastic microtiter plates for 90 min at 37°C in 5% CO₂. The nonadherent population was removed by vigorous washing with a Pasteur pipette.

Irradiation. Cells were irradiated from a ¹³⁷Cs source at a rate of 170 rad/min.

Th1 and Th2 HTL Clones. The derivation and characterization of a panel of OVA-specific, I-A^k-restricted Th1 (designated GL 15, GL 17, and GL 18) and Th2 clones (designated L 16, L 17, and L 18) have previously been described (7). The Th1 clones secrete IFN- γ , IL-2, and IL-3 and the Th2 clones secrete IL-3 and IL-4 when activated (7). The conalbumin-specific Th2 clone D10.G4.1 was kindly provided by Dr. C. Janeway, Jr. (Yale University, New Haven, CT). The fowl gamma-globulin-specific Th2 clone FGG3 (8) and anti-IE^k autoreactive Th2 clone L (9) were kindly provided by Dr. J. Quintans (The University of Chicago).

Lymphokine Assays. IL-4 concentration was determined using a subclone of HT-2 cells (10) that respond well to both IL-2 and IL-4. Identity of stimulatory activity was confirmed by blocking with mAbs specific for murine IL-2 and IL-4, used alone or in combination (11). Activities were compared with those of recombinant standards for calculation of concentration, expressed in U/ml. IL-3 activity was assessed as described (11).

Proliferation Assays. Cloned Th1 and Th2 cells (5×10^4) were cultured for 42 h with irradiated (2,000 rad) hepatic NPC or splenic cells (3×10^3 to 3×10^6) and antigen in 200 μ l total volume at 37°C in 96-well flat-bottomed microtiter plates (3596; Costar, Cambridge, MA). In selected experiments, rIL-1 (100 U/ml), rIL-6 (2,000 U/ml), and rIL-7 (1,000 U/ml), alone or in combination, were added at the beginning of the cultures. The cultures were pulsed with 1 μ Ci of [³H]TdR (New England Nuclear, Boston, MA) and harvested 6 h later using a 200-A PhD cell Harvester (Cambridge Technology, Cambridge, MA). Radioactivity was assessed by liquid scintillation spectrometry (LS-230; Beckman Instruments, Inc., Fullerton, CA). Each assay result is the mean of three replicate cultures \pm SD.

Results and Discussion

A panel of OVA-specific, H-2^k-restricted Th1 and Th2 HTL clones have recently been derived (7). Since these clones react to the same antigen, they represent an ideal system for investigating differences in antigen presentation among disparate accessory cell populations. To determine if hepatic accessory cells were as capable as splenic accessory cells in stimulating the proliferation of Th1 and Th2 HTL, irradiated splenic cells and hepatic NPC freshly isolated from C3H/HeN or B10.A mice were cultured for 48 h with several Th1 and Th2 clones in the presence of antigen. As shown in Fig. 1, splenic cells stimulated the proliferation of both Th1 and Th2 clones. In contrast, hepatic NPC stimulated only the proliferation of Th1 and not Th2 clones.

In subsequent experiments, only minimal proliferation of Th2 HTL clones was observed despite a wide region of antigen concentrations (200–800 μ g/ml) and he-

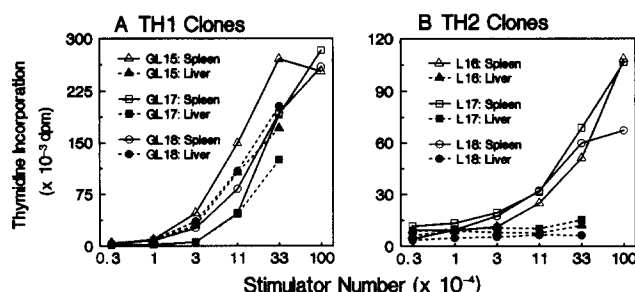


FIGURE 1. Stimulation of Th1 and Th2 clones proliferation by splenic cells and hepatic non-parenchymal cells Th1 and Th2 clones (5×10^4 cells) were cultured with OVA ($200 \mu\text{g/ml}$) plus irradiated splenic cells (*open symbols*) or hepatic NPC (*closed symbols*) from the same C3H/HeN mice. Thymidine incorporation was measured at 48 h, and the results are expressed as the mean dpm of triplicate cultures.

patic NPC (3×10^3 to 1.5×10^6 cells). Maximum proliferation of Th1 cells was reached with 10^6 hepatic NPC and of Th2 cells with 5×10^5 splenic cells; higher cell concentrations usually resulted in suboptimal Th1 and Th2 proliferation. Adherent hepatic NPC were also assayed for their ability to stimulate Th1 and Th2 clones in the presence of antigen. As with the unfractionated NPC, the adherent cell population stimulated the proliferation of Th1, but not Th2 clones (data not shown). In contrast to splenic cells, hepatic NPC did not stimulate the proliferation of other H-2^k-restricted Th2 clones derived in other laboratories, including the conalbumin-specific D10.G4.1, the fowl gammaglobulin-specific FG3 (8), and the autoreactive clone L (9) (data not shown).

The requirements for HTL proliferation by APC in response to antigenic stimulation include: (a) the uptake and degradation of the antigen by the APC; (b) the interaction of the complex of antigen peptide/class II MHC molecule with the TCR; (c) the production of cofactors (e.g., IL-1 and IL-6) by APC. The inability of hepatic NPC to stimulate the proliferation of Th2 clones could be the consequence of a defect of any of these APC functions.

Th2 HTL have been reported to produce IL-3, IL-4, and IL-5 (6). Moreover, IL-4 is required for autocrine growth (12). We reasoned that if hepatic APC are able to process and present antigen to Th2 clones, lymphokines should be detectable in the supernatants derived from those cultures. 10^5 – 10^6 irradiated hepatic NPC or splenic cells were cultured with Th2 clones in the presence of OVA. After 24 h, the supernates were harvested and assayed for IL-4. As shown in Table I, despite their

TABLE I
IL-4 Production of Th2 Clones in Response to Liver Cells or Spleen Cells

Th2	Liver cells (3×10^3)	Spleen cells (3×10^5)
	<i>U/ml</i>	
L16	1,195	1,935
L17	505	1,195
L18	875	2,110

Irradiated (2,000 rad) liver cells were cultured with 5×10^4 Th2 clones in complete media. The supernates were harvested at 24 h. IL-4 activity of supernates was determined by using an IL-4-responsive HT-2 cell line. Identity of IL-4 was confirmed by blocking with mAb specific for murine IL-4.

inability to support proliferation of Th2 clones, hepatic NPC, in the presence of antigen, stimulated Th2 cells to produce IL-4. Similar results were observed with IL-3 production (data not shown). Although quantitatively less IL-4 was produced by Th2 clones stimulated by hepatic NPC than by splenic cells, these results suggest there was engagement by MHC/peptide on the hepatic APC with the TCR on the Th2 cell.

Another possible explanation for the negligible Th2 proliferation is a deficiency of hepatic NPC in the production of a secreted or membrane-associated cofactor. IL-1 has been reported to augment the proliferation of Th2 but not Th1 clones (13). Moreover, IL-6 and IL-7 also appear to have cofactor activity for T cell proliferation (14, 15). We next examined the possible synergistic effect of rIL-1, rIL-6, and rIL-7 on the stimulation of proliferation of Th2 clones by hepatic NPC and antigen. As shown in Fig. 2, none of these ILs, alone or in combination, augmented the minimal proliferation of the Th2 clone L18 by hepatic NPC plus OVA. Similar results were observed with several other Th2 clones (data not shown).

Hepatic NPC represent a heterogeneous population of cells, which include CD4⁺ and CD8⁺ lymphocytes, Kupffer, Ito, endothelial, and NK cells. It is conceivable that a subset of NPC may be inhibiting the accessory cells that stimulate Th2 cells, perhaps by secretion of IFN- γ (16). To test this hypothesis, several Th2 clones were cultured with irradiated spleen cells, OVA, and hepatic NPC (adherent, nonadherent, or unfractionated) at a wide range of concentrations. As shown in Fig. 3, Th2 cells proliferated well in response to spleen cells and OVA alone; this Th2 response was inhibited only modestly by the addition of the highest concentration of hepatic NPC. Therefore, the inability of hepatic NPC to stimulate Th2 cell proliferation does not appear to be caused by an inhibitory factor produced by NPC.

Macrophages, dendritic cells, B cells, Langerhans cells, and endothelial cells are all capable of presenting antigen in a secondary immune response in which primed T cells or T cell lines are used as responders. However, because Th1 and Th2 subsets have different growth factor requirements, cell surface markers, and possibly activation pathways, it is conceivable that a specific accessory cell (e.g., macrophage or B cell) may present antigen more effectively to Th2 than to Th1 cells. Indeed, the liver has quantitatively fewer B cells but a greater number of macrophages than the spleen. Therefore, to account for the poor stimulation of Th2 proliferation, we speculate that the murine liver either lacks a specific accessory cell that enables Th2 cells to proliferate or fails to produce an unknown cofactor required for Th2 proliferation.

The stimulation of Th1 but not Th2 cell proliferation by hepatic NPC may have functional significance. Although the liver is the major site for the removal and degra-

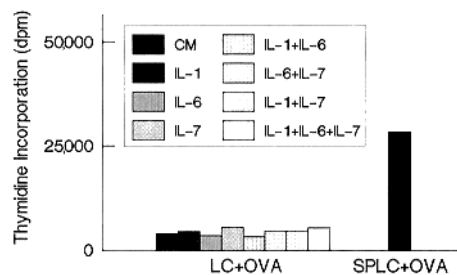


FIGURE 2. IL-1, IL-6, and IL-7 are not cofactors for the proliferation of Th2 clones by hepatic non-parenchymal cells. The Th2 clone L18 (5×10^4 cells) was cultured with OVA (200 $\mu\text{g}/\text{ml}$) and irradiated hepatic NPC or splenic cells (5×10^5) from the same C3H/HeN mice with complete media (CM) or with rIL-1 (100 U/ml), rIL-6 (2,000 U/ml), and rIL-7 (1,000 U/ml), alone or in combination. Thymidine incorporation was measured at 48 h, and the results are expressed as the mean dpm of triplicate cultures.

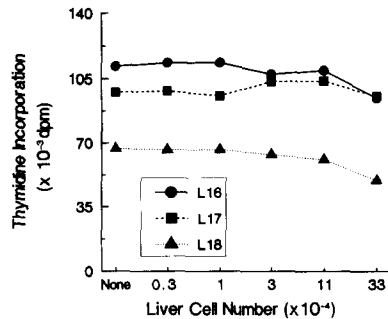


FIGURE 3. Hepatic nonparenchymal cells do not inhibit the proliferation of Th2 clones. Th2 clones (5×10^4 cells) were cultured with irradiated C3H/HeN spleen cells (5×10^5 cells) plus OVA ($200 \mu\text{g/ml}$) with or without the addition of irradiated hepatic NPC from the same animal. Thymidine incorporation was measured at 48 h, and the results are expressed as the mean dpm of triplicate cultures.

dation of antigens that have entered the portal venous circulation from the gastrointestinal tract, humoral responses to orally administered antigens are rare. Moreover, specific immunologic tolerance (4) and T cell suppression (5) after intragastric or intraportal administration of both soluble and particulate antigens have been reported. The mechanisms underlying this immunologic unresponsiveness, often referred to as the Chase-Sulzberger effect, are unknown. Our observations suggest that selective activation of Th1 cells, resulting in inhibition of specific antibody production, could account for the absence of humoral immune responses to orally administered antigens. The implications of these findings remain to be explored in vivo.

Summary

The liver is the major site of clearance and degradation of foreign antigens from the portal circulation. Despite the presence of hepatic accessory cells, antibody responses to orally administered antigens are uncommon. To ascertain if hepatic accessory cells are incapable of stimulating specific subsets of T lymphocytes, freshly isolated hepatic nonparenchymal and splenic cells were cultured with a panel of antigen-specific, H-2-restricted Th1 and Th2 HTL clones. Whereas spleen cells stimulated the proliferation of both Th1 and Th2 clones, hepatic nonparenchymal cells (NPC) stimulated the proliferation of only Th1 and not Th2 clones. Adding rIL-1, rIL-6, and rIL-7, alone or in combination, to the cultures did not result in proliferation of the Th2 clones. Despite the absence of Th2 proliferation, NPC were able to stimulate the secretion of IL-3 and IL-4 by Th2 clones in the presence of antigen. Moreover, adding hepatic NPC did not inhibit spleen cells from stimulating Th2 clones in the presence of antigen. Thus, the inability of liver cells to stimulate the proliferation of Th2 helper T lymphocytes appears to be secondary to an absence of either an unknown accessory cell cofactor or an accessory cell that preferentially presents antigen to Th2 cells. The selective activation of Th1 and not Th2 cells by liver accessory cells may result in suppression of antibody responses to orally administered antigens.

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