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Organ-Specific Cytokine Polarization Induced by Adoptive Transfer of Transgenic T Cells

Lei Zhang,* Elaine F. Lizzio,* Elena Gubina,* Trina Chen,* Howard Mostowski,† and Steven Kozlowski1*

There are two distinct phenotypes of T cell cytokine responses that lead to different effector functions and different outcomes in disease processes. Although evidence suggests a possible role of the local microenvironment in the differentiation or localization of T cells with these phenotypes, there are no examples of divergent T cell cytokine phenotypes with the same Ag specificity concurrently existing in different tissue compartments. Using a CD8+ T cell adoptive transfer model for graft-vs-host disease, we demonstrate that a potent type 2 cytokine response develops in the spleen while a potent type 1 cytokine response simultaneously develops in the testis. These experiments demonstrate for the first time that cytokine production can be oppositely polarized in different organs of the same individual. This may have important implications for organ-specific pathology in infection or autoimmunity: infections or autoimmune diseases that affect multiple organs may have heterogeneity in tissue cytokine responses that is not revealed in systemic lymphocyte cytokine responses. Therefore, attempts to modulate the immune response phenotype may ameliorate pathology in one organ while exacerbating pathology in another. The Journal of Immunology, 2002, 169: 5514–5521.

The Th1- and T cytotoxic (Tc) subsets of T cells produce type 1 cytokines, such as IFN-γ and TNF-β, while the Th2 and Tc2 subsets produce type 2 cytokines, such as IL-4, IL-5, and IL-10 (1, 2). Because the differentiation of an immune response to a type 1 or type 2 cytokine response affects the outcome in disease models (3) and plays a role in human disease (4), it is important to understand the signals that control this differentiation.

Recent studies on the mechanisms of T cell differentiation suggest that epigenetic changes in regions containing cytokine genes are critical (5). Master genes controlling these changes may include GATA-3 for type 2 cytokine differentiation and T-bet for type 1 cytokine differentiation (6, 7). Many other molecules play a role in this differentiation, including c-Maf, JunB, NFAT, STAT4, and STAT6 (6, 7). The nuclear transport of STAT transcription factors is driven by cytokines. Thus, the role of cytokines in signals for T cell differentiation is not surprising. Although many factors have been found to influence the selective differentiation of T cells, the cytokine environment during initial T cell priming is a major factor in deciding between the Th1- or Th2-type differentiation pathway (3, 8). The presence of elevated levels of IL-12/IFN-γ or IL-4 during the early T cell priming will result in differentiation toward type 1 or type 2 cytokine responses, respectively. This sets up a positive feedback loop for the type 1 or type 2 response. In addition, type 2 cytokines can inhibit differentiation to, or cytokine production by, type 1 T cells and vice versa (9, 10). The combination of a positive feedback loop with this inhibitory cross-regulation can push a minor tilt toward one phenotype into a fully polarized response. Trafficking of polarized T cells or systemic levels of cytokines could then spread the response phenotype throughout the entire organism (11). Such systemic cytokine polarization can be seen in a number of disease models; however, immune responses can also have a mixed phenotype. Both polarized T cell phenotypes have been shown to coexist in normal human PBLs (12) and in disease states (13, 14). If polarization of T cells always occurs as a systemic phenomenon, consistent mixed phenotypes would be unlikely, because a small change in initial differentiation or cytokine environment could have a profound impact on outcome. However, either the compartmentalization of T cell development in local microenvironments or the selective trafficking of T cell phenotypes to protected local environments would be compatible with stable mixed cytokine responses.

Local microenvironments may have levels of cytokines that are different from those in the systemic circulation or other tissues. Recent studies of IL-4R and STAT knockout mice (15–17) suggest that there are factors other than cytokines that can also drive T cell differentiation. Ag dose or avidity (18), costimulatory molecules (19), chemokines (20), and APC (21–23) have all been implicated as modulators of T cell polarization. All of these factors may be different in specific tissues or microenvironments.

There is also direct evidence that T cell differentiation is dependent on the local microenvironment. Studies of T cell priming in the pulmonary airways suggested that the lung environment per se is responsible for Th2-biased T cell differentiation (24). Ag priming in immune-privileged tissues can lead to altered T cell differentiation (25–27). Although these studies suggest the local environment can affect initial T cell differentiation, the phenotypes do not remain compartmentalized. The above studies show priming of the lung environment leads to a systemic Th2 bias, and priming of immune-privileged tissue leads to a systemic immune deviation. Even though compartmentalization of different response phenotypes would be a useful explanation for mixed responses, there is no clear evidence for the coexistence of oppositely polarized microenvironment-specific phenotypes in a single immune response.

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2 Abbreviations used in this paper: Tc, T cytotoxic; GVDH, graft-vs-host disease; MCP-1, monocyte chemoattractant protein-1.
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The only way to establish the coexistence of such compartmentalization is through in vivo studies of the T cell phenotype in different tissues.

In vivo studies of T cell cytokine polarization have generally evaluated polyclonal responses (18), and these studies have led to the development of one response phenotype or the other. Adoptive transfers of TCR transgenic CD4+ T cells have been used to visualize T cell localization and activation (28). Although such experiments have shown that effector cells migrate to nonlymphoid tissues and produce increased amounts of IFN-γ, no evidence for Th2 T cells in any tissue is presented (29). CD8+ memory effector T cells have also been shown to preferentially localize to nonlymphoid tissues (30) but, as with CD4+ T cell models, no type 2 cytokine responses were shown. Although the cytokine phenotype of CD8+ T cells can influence their localization through migration (31), simultaneous compartmentalized type 1 and type 2 cytokine production in a single immune response has not been demonstrated. To evaluate whether type 1 and type 2 responses could concurrently evolve in different locations from a single immune response, we chose a transgenic CD8+ T cell graft-vs-host disease (GVHD) model. Because GVHD is a multorgan process, it was likely that there would be T cell activation at multiple sites for histological evaluation. Although much of the data on T cell differentiation has been generated in studies of CD4+ T cells, CD8+ T cells can differentiate into type 2 as well as type 1 cytokine-producing effectors (2), and CD8+ T cell differentiation is influenced by cytokines in a manner similar to that of CD4+ T cells. Evaluation of a CD8+ T cell GVHD model revealed organ-specific cytokine polarization associated with organ-specific differences in a chemokine and chemokine receptors. The clonotypic TCR of the transferred transgenic cells demonstrates that the organ-specific differences are not due to clonal variations in the TCR-Ag interactions.

Materials and Methods

Peptides

Peptides were obtained from the Center for Biologics Evaluation and Research Facility for Biotechnology Resources (Bethesda, MD). Peptides were synthesized on ABI 433 peptide synthesizer (Applied Biosystems, Foster City, CA). The peptide purity was determined to be >99.5% by capillary electrophoresis (PACE 5000, Beckman Coulter, Fullerton, CA). The following peptides were used in this study: p2C-QY5, QLSPYFPDL; and p2C-A3, LSAFPFDL.

Antibodies

Primary Abs.

The following primary Abs were obtained from BD PharMingen (San Diego, CA): monoclonal rat anti-mouse B220, IL-4, IL-5, and IFN-γ Abs. Purified monoclonal anti-clonotypic 2C TCR Ab (1B2) (32) was generously provided by Dr. D. Margulies (National Institutes of Health). B6D2 F1 mice (aged 8–12 wk) were immunized i.p. 1 day later with 10 μg of synthetic peptide prepared in PBS and injected alone or emulsified in CFA (Sigma-Aldrich). Organs and tissues were harvested 4 days after immunization for immunohistochernistry.

Cytokine ELISA

Sera or culture supernatants were assayed for detection of IL-4 and IFN-γ with a commercially available kit (Endogen, Boston, MA) according to the manufacturer’s directions. HRP-conjugated streptavidin (Zymed, San Francisco, CA) and tetramethylbenzidine (Dako) were used in the cytokine ELISAs. The absorbance was read on a Bio-Rad (Hercules, CA) model 3550 microplate reader at 655 nm.

Immunohistochemistry

Harvested tissues and organs were prefixed in 4% paraformaldehyde for 4–8 h and then snap-frozen in liquid nitrogen. The tissues were either stored at −80° C for later use or immediately embedded in OCT embedding medium (Sakura, Torrance, CA). Cryostat sections (5 μm) were cut at −20° C and collected onto slides. The sections were air-dried and then fixed, using a graded series of acetone solutions in water (60, 70, 80, and 90% acetone; 3 min per solution). The sections were either air-dried overnight and then stored at −20° C or rehydrated for immunostaining. An avidin-biotin-peroxidase complex (Vector, Burlingame, CA) method was used for the immunohistologic staining of the frozen sections as described previously (35). Tissue sections from normal untreated mice or tissue sections from adoptively transferred mice stained with control primary Abs were used as negative controls.

Primary Abs were diluted at 1–10 μg/ml in freshly prepared 2% casein solution. Normal goat, rabbit, and rat IgG controls were prepared in the same manner. Sections were first incubated with a 2% casein solution in PBS for 2 h before addition of primary Abs. After primary Ab incubation for 2–4 h, the endogenous peroxidase activity was inactivated by 5% hydrogen peroxide in 0.1% sodium azide for 10 min. Secondary Abs were prepared in 2% casein solution at a concentration of 1–10 μg/ml and incubated with section slides for at least 2–4 h, and then washed in 0.5% Tween 20 detergent in PBS for 5 times for 3 min per wash. The avidin-biotin-peroxidase complex was then prepared and used according to the ABC kit protocol (Vector Laboratories). After 2 h of incubation, the peroxidase reaction was developed in 0.05% 3,3′-diaminobenzidine tetrahydrochloride, and the sections were counterstained with Gill’s hematoxylin no. 3 (Sigma-Aldrich). For staining 2C T cells, the FITC-labeled mAb (1B2) was used as the primary Ab and followed by biotinylated rabbit anti-FITC Ab and avidin-biotin-peroxidase. For staining of B220, IL-4, IL-5, and IFN-γ, the biotinylated anti-rat IgG was used as the secondary Ab. For staining of CCR4, CCR5, and MCP-1, the biotinylated anti-goat IgG was used as the secondary Ab.

For double-staining of tissue sections after DAB color development, the slides were washed in 0.5% Tween 20 detergent in PBS five times and quenched again with 5% hydrogen peroxide in 0.1% sodium azide for 10 min. Tissue sections were then incubated with Avidin D (Vector Laboratories) blocking solution for 15 min. After rinsing with PBS, the sections were incubated with biotin blocking solution for 15 min. Then the avidin-biotin-peroxidase complex method as described above was performed with a secondary primary Ab until the color development step. The Vector SG substrate kit for peroxidase was then used for color development. To 5 ml of PBS, 3 drops of chromogen and 3 drops of hydrogen peroxide solution were added. This solution was immediately applied to the tissue sections and incubated for 1–2 min. The sections were then counterstained with Gill’s hematoxylin no. 3. Single staining for B220 was also developed with the Vector SG substrate.
In situ apoptosis detection

Tissue sections were fixed, as described in Immunohistochemistry. The apoptosis detection kit was obtained from R&D Systems. Apoptosis detection was performed according to the manufacturer’s protocol. Briefly, tissue sections were incubated with proteinase K (R & D Systems) solution for 10 min. After washing in PBS three times, the tissue sections were incubated with quenching solution for 5 min. Then, the tissue sections were covered with a labeling reaction mixture for 1 h at 37°C. The in situ labeling was stopped by incubating sections with reaction stop buffer for 5 min and then followed by incubation with biotinylated anti-BrdU Ab for 2 h. After washing in 0.5% Tween 20 PBS, the sections were incubated with streptavidin-HRP solution for 10 min. The peroxidase reaction was developed in diaminobenzidine, and then the tissue sections were counterstained with Gill’s hematoxylin no. 3.

Ex vivo cytokine detection of cells from spleen and testis

B6D2 F1 mice received an adoptive transfer of 2C T cells and were immunized with p2C-QY5 peptide as described above. Four days after immunization, the host B6D2 F1 mice were sacrificed and cell suspensions derived from spleen or testis were cultured in complete medium (RPMI 1640 (BioWhittaker, Walkersville, MD) plus 100 U/ml penicillin/streptomycin, nonessential amino acids, 2 mM l-glutamine, and 50 mM 2-ME) with 5% FBS for 24 h. Supernatants from the cultures were harvested and assayed for IL-4 and IFN-γ production by cytokine ELISA as described above.

Results

In vivo activation of adoptively transferred 2C T cells

The TCR cloned from the alloreactive 2C cell line recognizes the peptide p2Ca (36) in the context of the major histocompatibility molecule H-2Ld (3). This peptide is derived from endogenous 2-oxoglutarate dehydrogenase (37). The binding affinity of the 2C TCR for the p2C/H-2Ld complex has been measured (38, 39), and many variants of the p2Ca peptide have been studied (40, 41). T cells, transgenic for the 2C TCR, are strongly biased toward production of type 1 cytokines in response to p2C peptide/H-2Ld stimulation in vivo experiments (34). We confirmed the in vitro studies with the potent p2C variant peptide p2C-QY5 and with the less potent p2C-A3 variant peptide, both in the absence and presence of IL-4.

FIGURE 1. Engraftment and tissue effects of the adoptively transferred 2C TCR transgenic T lymphocytes. B6D2 F1 mice that received 2C T cells and were immunized with p2C-QY5 in CFA, as described in the Materials and Methods, displayed clonotypic receptor positive 2C T cells (red-brown staining) in spleen (a), testis (b) and lymph node (c). Spleen and testis from five mice and lymph node from two mice were examined at ×400 magnification. Apoptotic cells in the interstitium of testis (d) were detected by in situ apoptotic labeling (brown staining), as described in Materials and Methods (×1000 magnification). Reduction in the number of white pulp B cell zones in spleen sections of adoptively transferred and immunized B6D2 F1 mice (e) compared with that of normal spleens (f) was observed by immunohistochemical staining of the B cell marker B220 (dark blue staining). The photographs of B220-stained sections were taken at ×100 magnification. The pictures represent immunohistochemical staining typical of multiple experiments. To evaluate the role of peptide immunization, 2C T cells were adoptively transferred into B6D2 F1 mice, and mice were immunized as indicated (g). The engraftment of 2C T cells was examined by immunohistochemistry for the clonotypic receptor as per above. The numbers of cells expressing 2C TCR in three noncontiguous ×400 microscopic fields were counted for each group of mice (n = 3–5; n = 2 for lymph node) and presented as means ± SEM.

FIGURE 2. Organ-specific type 1 and type 2 cytokine profiles were observed in tests and spleen, respectively. B6D2 F1 mice that received 2C T cells and an immunization with p2C-QY5 peptide in CFA were examined by immunohistochemistry, as described in the Materials and Methods. Large numbers of cells producing IL-4 (b) but no IFN-γ (a) were observed in spleen, while large numbers of cells producing IFN-γ (d) but no IL-4 (c) were also identified in testis of the same mice. Without peptide immunization, no IL-4 (e) or IFN-γ (f) producing cells were detected in spleen or testis, respectively. The pictures represent immunohistochemical staining typical of multiple adoptive transfers. Organs from five mice were examined at ×400 magnification.
hosts to prevent host-vs-graft responses by expression of H-2 b and

and adoptively transferred into host mice, and

or IFN-γ/H9253

testis (Fig. 2, a–c). Considerable numbers of 2C T
cells were found in spleen and testis, and somewhat lower numbers
of 2C T cells were found in lymph nodes of mice challenged with
p2C-QY5 (Fig. 1g). B6D2 F1 mice receiving 2C T cell adoptive
transfer without peptide challenge had only small numbers of 2C
T cells in the spleen and even fewer in the testis (Fig. 1g). Similar
results were also seen with p2C-A3 peptide challenge.

Although we demonstrate engraftment of large numbers of
adoptively transferred cells into the spleen and testis of host ani-

tals in our GVHD model, GVHD implies tissue damage. To as-

ess this, we evaluated apoptosis in the testis and B cell popula-
tions in the spleen. Testis was stained for apoptosis, and significant
apoptosis was present in the interstitium (Fig. 1d). Although many
of these cells may be the infiltrating 2C T cells, some of the apo-
ptotic cells have the triangular morphology and eccentric nuclei of
testicular Leydig cells. This suggests the 2C T cells are causing
host tissue damage. In the spleen, we found fewer apoptotic cells
data not shown). Both peptides led to type 1 cytokine production
in vitro, even though the p2C-A3 peptide is 30-fold less effective
at sensitizing H-2Ld targets than is the p2C-QY5 peptide (40, 41).

To evaluate the in vivo response of unprimed 2C T cells, these
cells were purified and adoptively transferred into host mice, and
the mice were then immunized with p2C-QY5 peptide. The adap-
tive transfer experiments were performed in B6D2 F1 (H-2bd)
hosts to prevent host-vs-graft responses by expression of H-2b and
to provide an H-2b background for expression of H-2Ld molecules
necessary for presentation of p2C. Although the level of endoge-
uous p2C peptides in the H-2Ld-expressing host is sufficient to
stimulate a graft-vs-host response (42), we immunized the host
with p2C peptides after adoptive transfer to increase the level of T
cell activation. Because in our previous in vitro experiments 2C T
cell proliferation and cytokine production peaked after 4–5 days
of incubation with plate-coated H-2Ld MHC and p2C-QY5 peptide
(34), we sacrificed the mice on the fourth day after peptide chal-

lenge. Evaluation of serum for cytokines at that time revealed a
mixed response with both IFN-γ (65.2 ± 15.9 ng/ml) and IL-4
(11.5 ± 0.6 ng/ml) by ELISA.

Localization and tissue effects of engrafted 2C T cells

To evaluate the tissues in which the 2C TCR T cells have local-
ized, we performed immunohistochemical staining with the 2C
clonotypic TCR-specific Ab, 1B2 (32). A variety of different or-
gans and tissues were examined, including heart, liver, kidney,
skeletal muscle, spleen, mesenteric lymph node, testis, and brain.
Among these organs and tissues, only spleen, testis, and lymph
node were found to have 2C T cell infiltration by immunohisto-
chemistry, as shown in Fig. 1, a–c. Considerable numbers of 2C T
cells were found in spleen and testis, and somewhat lower numbers
of 2C T cells were found in lymph nodes of mice challenged with
p2C-QY5 (Fig. 1g). B6D2 F1 mice receiving 2C T cell adoptive
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of these cells may be the infiltrating 2C T cells, some of the apo-
ptotic cells have the triangular morphology and eccentric nuclei of
testicular Leydig cells. This suggests the 2C T cells are causing
host tissue damage. In the spleen, we found fewer apoptotic cells
data not shown); however, a lower frequency of white pulp B cell
areas was noted in some experiments (Fig. 1, e and f).

Organ-specific cytokine polarization is induced by engrafted
2C T cells

To evaluate the nature of the T cell responses in different tissues,
immunohistochemistry for type 1 and type 2 cytokines was per-
formed on spleen and testis, the principal sites of T cell engraft-
ment. Mice, adoptively transferred with 2C T cells and immunized
with p2C-QY5 in CFA, had almost no IFN-γ-producing cells but
large numbers of IL-4-producing cells in the spleen, suggesting a
type 2 polarized cytokine response (Fig. 2, a and b). In contrast,
these animals had IFN-γ- but almost no IL-4-producing cells in the
testis (Fig. 2, d and e). Very few IL-4-producing cells in the spleen
or IFN-γ-producing cells in the testis were detected in B6D2 F1
mice that were adoptively transferred with 2C T cells but received
no peptide challenge (Fig. 2, c and f). Fig. 3a shows counts of IL-4,
IL-5, and IFN-γ cytokine-producing cells in spleen and testis after
different immunization conditions, including in the absence of
CFA and with the weaker p2C-A3 peptide. CFA treatment alone
did not induce the response of either organ, demonstrating that the
Ag, not the adjuvant, drives the observed responses.

We verified the organ-specific cytokine polarization by cytokine
ELISA. Host mice adoptively transferred with 2C T cells and im-
munized with peptide were sacrificed, and cell suspensions of the
testis and spleen were cultured. The culture supernatants were
tested by ELISA for IL-4 and IFN-γ by cytokine ELISA, as de-
scribed in Materials and Methods. The numbers of cells producing cytokines in three noncontiguous 400 microscopic fields were counted for each group of mice (n = 3–5) under each experimental condition and presented as means ± SEM. b, IL-4 and
IFN-γ were detected in cultures of spleen and testis, respectively. Spleen and testis of B6D2 F1 mice that received an adoptive transfer of 2C T cells and an immunization with p2C-QY5 peptide were harvested, and the cell suspensions derived from these organs were cultured for 24 h. The super-
natants were then assayed for IL-4 and IFN-γ by cytokine ELISA, as
described in Materials and Methods. The figure represents the average of data from three B6D2 F1 mice, and the error bars indicate the SD.

There is a difference between spleen and testis in expression of
the chemokine MCP-1 and chemokine receptors

To confirm and extend our findings of organ-polarized type 1 and
type 2 cytokine responses, we evaluated spleen and testis in situ
staining for the chemokine MCP-1 and the chemokine receptors CCR4 and CCR5. Th1 and Th2 CD4+ T cells have been found to preferentially express CCR5/CXCR3 and CCR3/CCR4 chemokine receptors, respectively (43, 44). Similarly, Tc2 and Tc1 subsets of CD8+ T cells predominantly express CCR4 and CCR5, respectively (31). Mice deficient in MCP-1 failed to mount a Th2 response (20). Thus, if in our model the tissues are oppositely polarized, we would expect to find tissue-specific changes in MCP-1 or chemokine receptors. Consistent with our cytokine data, a large number of MCP-1-producing cells and cells expressing CCR4 were found in the type 2 polarized spleen (Fig. 4, a and b) but not in the testis of our peptide-immunized GVHD model (Fig. 4, d and e). The CCR4-expressing cells are mainly found in the white pulp, while cells expressing MCP-1 are found in the red pulp. Also, as expected, moderate numbers of CCR5-expressing cells were found in the type 1 polarized testis (Fig. 4f), and only small numbers were found in spleen (Fig. 4c). The counts of positively stained cells under different immunization conditions are shown in Fig. 5.

The organ-specific tissue phenotypes are reflective of 2C T cell organ-specific polarization

In the spleen, both clonotypic TCR and IL-4 positive cells primarily reside in the red pulp, and fewer of these cells reside in the white pulp. In the testis, both clonotypic TCR and IFN-γ positive cells reside at the periphery of the tubules or in the interstitium. However, the similar distribution of clonotypic TCR and cytokine positive cells does not prove that the 2C T cells are the direct source of the cytokines. It remains possible that host-derived cells are responsible for the observed cytokines and chemokine receptor expression. To demonstrate that 2C T cells are expressing the tissue-specific cytokines and chemokine receptors, we double-stained the tissues for clonotypic TCR and cytokines or chemokine receptors. Splenic sections were stained for clonotypic TCR and IL-4 (Fig. 4g) or clonotypic TCR and CCR4 (Fig. 4h). Although isolated staining for IL-4 was noted, many splenic cells displayed the black staining typical of dual positivity. Splenic cells were also double positive for clonotypic TCR and CCR4. Sections of testis were stained for clonotypic TCR and IFN-γ (Fig. 4i) or clonotypic TCR and CCR5 (Fig. 4j). Cells were noted to have dual staining for clonotypic TCR and both IFN-γ and CCR5. These results demonstrate that, in addition to being a critical factor in driving the organ-specific polarization, the 2C T cell are themselves polarized and a direct source of the cytokines observed.

![FIGURE 4](http://www.jimmunol.org/content/5518/13/5518/F4)

**FIGURE 4.** Organ-specific CCR5 and MCP-1/CCR4 were observed in testis and spleen, respectively. B6D2 F1 mice that received 2C T cells and an immunization with p2C-QY5 peptide in CFA were examined by immunohistochemistry as described in Materials and Methods. Large numbers of cells expressing MCP-1 (a) and CCR4 (b), but few CCR5 (c) positive cells, were observed in spleen. In testis of the same mice, large numbers of cells expressing CCR5 (f), but few cells expressing MCP-1 (d) or CCR4 (e), were identified. Organs from five mice were examined at ×1000 magnification. Immunohistochemical double-staining (×1000 magnification) revealed that 2C T cells in spleen and testis express the polarizing cytokines and chemokine receptors. B6D2 F1 mice that received 2C T cells and an immunization with p2C-QY5 peptide in CFA were examined by immunohistochemical double-staining, as described in Materials and Methods. Splenic tissue was stained for the 2C clonotypic receptor and IL-4 (g) or 2C clonotypic receptor and CCR4 (h). Tissue from testis was stained for the 2C clonotypic receptor and IFN-γ (i) or 2C clonotypic receptor and CCR5 (j). In the double-staining procedure, clonotypic receptor staining is yellow-brown, and cytokine or chemokine receptor staining is blue. Cells with dual staining have areas of black. The pictures represent immunohistochemical staining typical of multiple experiments.

![FIGURE 5](http://www.jimmunol.org/content/5518/13/5518/F5)

**FIGURE 5.** Organ-specific CCR5 and MCP-1/CCR4 were observed in testis and spleen, respectively. B6D2 F1 mice that received 2C T cells and an immunization with no Ag ( ), CFA alone ( ), p2C-A3 ( ), p2C-QY5 ( ), and p2C-QY5 + CFA ( ) were examined by immunohistochemistry for MCP-1, CCR4, and CCR5, as described in Materials and Methods. The numbers of cells in spleen and testis expressing MCP-1, CCR4, and CCR5 in three noncontiguous ×400 microscopic fields were counted for each group of mice (n = 3–5) under each experimental condition and presented as means ± SEM.
The organ-polarized cytokine responses are not due to the
differential activation and tissue migration of CD8+ and CD8−
2C T cells or due to T cell responses to other Ags

A percentage of 2C TCR transgenic T cells lack expression of CD8
cells that express other Vβ chains (46–48); however, the 2C TCR uses the Vβ8.2 chain (46). Therefore, an Mls response by the clonotypic 2C TCR is not a factor in the observed phenotype. However, to rule out any other possible confounding factors, such as a response by donor cells that express other Vβ chains reactive with Mls, other minor Ags in the B6D2 F1 host, or host hybrid resistance to parental H-2Dd, we performed the experiment with L3 mice as hosts. These mice are transgenic for H-2Ld on a B6 background that matches the background of the 2C TCR transgenic mice. Organ-specific polarization was also seen with adoptive transfer of 2C T cells into the L3 mice (Fig. 6), making the contribution of the host background genes extremely unlikely. Furthermore, the need for p2C peptide immunization to observe strong responses in either organ and the double immunostaining suggest that the p2C Ag and 2C TCR positive T cells are responsible for the responses seen in both the spleen and testis.

The organ-polarized cytokine responses are not limited to a
single time point

Because all our previous data for cytokine polarization was ob-
tained 5 days after 2C T cell transfer, we evaluated the spleen and testis of a B6D2 F1 host 10 days after transfer of 2C T cells and 9 days after peptide immunization with p2C-QY5 in CFA. Although the number of positively staining cells was somewhat decreased, cytokine polarization was still observed at this time, with IL-4 positive cells in the spleen and IFN-γ positive cells in the testis (Fig. 6).

Discussion

These results clearly demonstrate that, in our allogeneic 2C T cell transfer model, a single peptide immunization can activate its TCR transgenic T cells to produce type 1 and 2 cytokines in an organ-polarized fashion. Although a mixed type of cytokine production was observed in serum, organ-polarized type 1 and type 2 cytokine production was observed in tests and spleen, respectively.

Despite evidence suggesting a role of the local microenviron-
ment in the differentiation (24) of T cells, this is the first study showing simultaneous compartmentalized type 1 and type 2 cytokine responses to the same antigenic stimulus by clonotypic re-
ceptor T cells. The oppositely polarized responses were demon-
strated by both immunohistochemistry and ELISA on tissue cell suspensions. Double-staining of the tissues demonstrated that the clonotypic T cells themselves are positive for the IFN-γ in the testis and IL-4 in the spleen. Although we evaluated the cytokine-polarized tissues early after transfer and immunization, the tissues show evidence of differing pathologies that include splenic archi-
tecture changes and apoptosis in the tests. Thus, tissue-specific polarization may modify the pathology of multiorgan diseases such as GVHD.

Organ-specific cytokine polarization was also observed in ex-
periments controlling for CD8 positivity of the transferred cells and for the host background. Immunostaining for the chemokine MCP-1 and chemokine receptors, which correlate with cytokine phenotype, support the polarized cytokine results. Although it is attractive to postulate that locally expressed chemokines and/or chemokine receptors facilitate selective migration or differentiation, it is difficult to determine whether the organ-specific expres-
sion of CCR4/MCP-1 and CCR5 is the cause or the result of the organ-specific cytokine polarization. Staining of spleen and testis in normal mice did not reveal a detectable pretransfer difference in MCP-1, CCR4, CCR5, or chemokines (data not shown).

Many other factors may play a role in the observed tissue-spe-
cific effects. Ag dose or avidity (18) can alter T cell differentiation. We have used two different peptide ligands in our experiments, p2C-QY5 and p2C-A3. The sensitizing dose of peptide for 2C CTL lysis is 30-fold lower for the p2C-QY5 peptide than for the p2C-A3 peptide when presented on an H-2Ld positive target (40, 41). Despite a 30-fold difference in potency between the two pep-
tides, the organ-polarized phenotype is unaffected. Although it is possible that a greater difference in Ag potency or dose could alter the phenotype of the response, organ-specific cytokine polarization is not limited to a narrow range of Ag avidity.

The kinetics of Ag dose may also be relevant. The allogeneic transfer model can lead to some level of GVHD in the absence of immunization due to endogenous p2C Ag in the context of H-2Ld. Endogenous p2Ca/H-2Ld complexes would be present at a lower density than that induced by exogenous peptide immunization.
This lower antigenic stimulus would be present for the day between the transfer of cells and the peptide immunization in our model. It is possible that the lower level of stimulus can start driving a type 2 response in the spleen but be insufficient to do so in the testis. This may allow the more potent peptide immunization to drive a type 1 response in the testis but not in the type 2 committed spleen. Alternatively, the early lower level activation could have other important consequences, including altering migration patterns or facilitating selective lysis of APC. Such models suggest that immunopathology, in which there are rapid changes in the amount of Ag presented, could lead to organ- or tissue-specific compartmentalization of immune responses.

Certain tissue environments, such as the anterior chamber of the eye, the brain, and the testis have been described as immune-privileged. The presence of TGF-β (25) and the presence of Fas ligand (49) are two differences in these tissue compartments that have been described to modulate immune responses. It is of note that immune responses generated in these tissues can lead to systemic alteration of immunity rather than tissue-specific differences in immunity (27). Furthermore, immune-privileged environments often favor tolerance or type 2 cytokine responses (26). Thus, neither the type 1 cytokine phenotype noted in the testis nor the tissue-specific polarization we observed would be predicted by previous data from immune-privileged tissues.

In addition to possible local microenvironment differences in Ag density and kinetics, expression of Fas ligand, chemokines and cytokines, APC (21–23) and their costimulatory molecules (19) may also play a role in tissue-specific T cell polarization. Thus, the local microenvironment can operate through complex interactions involving many factors.

Although there is a specific combination of all these factors that promotes the different outcomes in the tissues we have evaluated, the interplay of all these factors make dissection of cause and effect difficult. This is illustrated by our data regarding MCP-1 and chemokine receptor expression. Whether these changes are the result or the cause of the tissue cytokine phenotype and whether they operate through selective effects on migration or differentiation will need to be addressed. Independent of the mechanistic details of our results, the tissue-specific differentiation and/or migration of cells with type 1 and 2 cytokine profiles, in response to clonotypic receptor T cells and a single antigenic peptide, demonstrate that the local microenvironment is a metamechanism for tissue-specific cytokine polarization.

This GVHD model has demonstrated compartmentalization of polarized type 1 and type 2 cytokine responses, despite a mixed systemic cytokine response. Although not all other multigran disease models with mixed cytokine phenotypes may be due to oppositely polarized microenvironments, it is critical to begin to evaluate the tissues in these models. Many of the factors that play a potential role in this GVHD model may be applicable to other situations. Clearly different tissues have different Ag presentation, chemokines, and cytokines. Infectious viruses may display different levels of Ag with different kinetics in different tissues. This suggests that infections or autoimmune diseases that affect multiple organs may also have heterogeneity in tissue cytokine responses. Therefore, attempts to modulate the immune response phenotype may ameliorate pathology in one organ while exacerbating pathology in another.

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References