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*J Immunol* 2009; 183:7547-7556; Prepublished online 4 November 2009; doi: 10.4049/jimmunol.0900519

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Unlike Th1, Th17 Cells Mediate Sustained Autoimmune Inflammation and Are Highly Resistant to Restimulation-Induced Cell Death

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Both Th1 and Th17 T cell subsets can mediate inflammation, but the kinetics of the pathogenic processes mediated by these two subsets have not been investigated. Using an experimental system in which TCR-transgenic Th1 or Th17 cells specific for hen egg lysozyme induce ocular inflammation in recipient mice expressing eye-restricted hen egg lysozyme, we found important differences in the in vivo behavior of these two subsets. Th1 cells initially proliferated considerably faster and invaded the eye more quickly than their Th17 counterparts, but then disappeared rapidly. By contrast, Th17 cells accumulated and remained the majority of the infiltrating CD4+ cells in the eye for as long as 25 days after transfer, mediating more long-lasting pathological changes. Unlike Th1, Th17 cells were highly resistant to restimulation-induced apoptosis, a major pathway by which autoimmune and chronically restimulated Th1 cells are eliminated. Th17 cells had reduced Fas ligand production and resistance to Fas-induced apoptosis, relative to Th1 cells, despite similar surface expression of Fas. Th17-induced ocular inflammation also differed from Th1-induced inflammation by consisting of more neutrophils, whereas Th1-induced disease had higher proportions of CD8 cells. Taken together, our data show that pathogenic processes triggered by Th17 lag behind those induced by Th1, but then persist remarkably longer, apparently due to the relative resistance of Th17 cells to restimulation-induced cell death. The long-lasting inflammation induced by Th17 cells is in accord with these cells being involved in chronic conditions in humans. The Journal of Immunology, 2009, 183: 7547–7556.

The recently described population of Th17 cells has been found to play major roles in defense against microbial infection (1–4), as well as in pathogenic autoimmune processes (5–9). The involvement of Th17 cells in human disease was particularly noted in chronic conditions (10, 11), including rheumatoid arthritis (12, 13), inflammatory bowel diseases (14, 15), multiple sclerosis (16, 17), psoriasis (11, 18), uveitis (19, 20), and chronic periodontal disease (21). Th17 cells were reported to be superior to Th1 in their capacity to adoptively transfer experimental autoimmune encephalomyelitis (EAE)4 (4, 6) or experimental colitis (22). Yet, highly purified Th1 have also been shown in several systems to be strongly immunopathogenic, capable of inducing disease in the CNS (23, 24) or the eye (25, 26).

Both Th1 and Th17 and their signature cytokines, IFN-γ and IL-17, respectively, are found at cell-mediated inflammatory sites, but there are conflicting reports concerning their specific roles in these processes. Thus, Kebir et al. (27) reported that Th17 cells are responsible for the breakdown of the blood-brain barrier in EAE, an essential early component for the pathogenic process of this disease. More recently, however, O’Connor et al. (24) reported that a portion of polarized EAE Th17 cells can switch phenotype to IFN-γ-producing cells, and that these IFN-γ-producing cells facilitate the CNS entry of EAE-inducing Th17 cells. The involvement of Th17 in the early phase of the pathogenic process was also indicated by Amadi-Obi et al. (20), who reported that the Th17 reached their highest level in mouse eyes developing experimental autoimmune uveitis (EAU) on day 14, with a gradual decline that was accompanied by an increase in Th1 levels. In contrast, however, Yoshimura et al. (28) reported that Th17 exert their immunopathogenic activity in EAU mainly at the later phase of the pathogenic process. Also, more recently, Hoyer et al. (29) reported that Th1 drive the early pathogenic response of the systemic autoimmune disease in IL-2-deficient mice, whereas Th17 cells are responsible for the chronic phase of this condition.

Many features of Th populations have been defined by using TCR-transgenic (Tg) mice in which most T cells express the same Ag receptor (26, 30, 31). Tracking the fate of TCR Tg T cells in vivo eliminates the possibility that changes in pathology or phenotype may be due to differences in affinity of the TCR, as seen in polyclonal T cell populations. We previously described a number of phenotypic differences between polarized Th1 and Th17 cells with specificity toward the same Ag, hen egg lysozyme (HEL) (26). Both lineages were found to be pathogenic, capable of inducing inflammation in eyes of Tg mice expressing HEL in their lens. The two lineages differed, however, in several key biological
features, including their capacity to stimulate lymphoid expansion in recipient mice, their expression of several surface markers in vitro and in vivo, their profile of chemokine secretion, as well as the ratio between donor and host cells at different time points of the inflammation process.

By tracking the fate of T cells in this model over time, we now report major differences between immunopathogenic Th1 and Th17 cells in terms of their proliferation and survival in vivo. Although Th1 HEL-specific donor cells initially proliferated more rapidly after transfer and invaded the recipient mouse eyes earlier, their numbers quickly declined in the eye after reaching the peak on day 5, and they were largely replaced by host T cells. In contrast, Th17 cells persisted for up to 25 days after transfer, leading to sustained ocular pathology. These kinetic differences were tied to a remarkable resistance of Th17 cells to apoptosis induced by antigenic restimulation in vitro, a failure related to reduced function of Fas, as well as reduced induction of Fas ligand (FasL). Resistance to apoptosis by Th17 cells may thus be responsible for the sustained T cell immunopathology mediated by this T cell subset. It is of note that Th17 cells are typically found in inflammatory lesions of Crohn’s disease, rheumatoid arthritis, and other chronic conditions listed above (10–21), suggesting that these cells play a major role in the pathogenic processes of chronic immune-mediated inflammation.

Materials and Methods

Mice

HEL-Tg mice expressing membrane-associated HEL under control of the αA-crystallin promotor, and on an FVB/N background, were generated as described (32). HEL-specific TCR-Tg mice (designated 3A9), on a B10.BR background, were a gift of M. Davis (Stanford University, Stanford, CA). Tg mice from each line were mated to produce (FVB/N x 10% of these cells stained for CD4.

Flow cytometric analysis of surface and intracellular molecules

Conventional methods were used for analysis of surface molecule expression.

For intracellular staining, reactivated Th1 and Th17 cell cultures were stimulated with 20 ng/ml PMA and 1 μM ionomycin (Sigma-Aldrich), plus Golgi-Stop (Molecular Probes), fixed, permeabilized using BD Cytofix/Cytoperm buffer (BrdU Flow Kit; BD Pharmingen), and stained with allophycocyanin-conjugated annexin V for 20 min and analyzed by flow cytometry after addition of propidium iodide. Specific cell death was calculated using the following formula: 1 – (% live, untreated cells)/(% live, treated cells).

Cell death assays

Cell death assays were performed on Th1- and Th17-polarized cells by incubating cells with plate-bound anti-CD3, or anti-Fas mixed with streptavidin in a 5:1 ratio, at the indicated concentrations, for 6–8 h, in complete medium containing 50 IU/ml IL-2. Cells were stained with allopbyocyanin-conjugated annexin V for 20 min and analyzed by flow cytometry after addition of propidium iodide. Specific cell death was calculated using the following formula: 1 – (% live, treated cells)/(% live, untreated cells), where cells that remained annexin V and propidium iodide negative were considered live cells.

For cell death analysis of transferred Th1 or Th17 cells, the cells collected from recipient eyes by physical measures were stained using anti-CD4 (PE), 1G12 (FITC), and allopbyocyanin-conjugated annexin V for 20 min, and analyzed by flow cytometry following addition of 7-amino-actinomycin D (7-AAD; BD Pharmingen).

BrdU incorporation assay

HEL-Tg recipient mice of reactivated Th1 or Th17 cells (5 × 10⁶) were injected i.p., on day 5 postcell transfer, with 1 mg of BrdU (BD Pharmingen). Recipient mice were euthanized 4 h later, and the infiltrating cells in their eyes were collected and stained with anti-CD4 (PE), anti-CD3 (PerCP-cy5.5), and 1G12 (FITC) Abs. BrdU incorporation was analyzed by flow cytometry in 1G12⁺ cell population.

FasL measurement

Th1 or Th17 cells were stimulated with cross-linked (plate-bound) anti-CD3 for 6–8 h, and induced FasL protein was detected using ELISA on cell supernatants (R&D Systems). For measurement of transcriptional level of FasL, in recipient eyes and Th1 and Th17 cells, total RNA was extracted and subjected to real-time PCR, using Fasl-specific probes for TaqMan

Adaptive transfer of reactivated cells and preparation of ocular single-cell suspensions

Polarized 3A9 Th1 or Th17 cells were harvested on day 3 of reactivation and resuspended in RPMI 1640, and 5 × 10⁷ Th1 or Th17 cells were injected via the tail vein into groups of naive HEL-Tg mice. At different time points postadoptive transfer, as indicated, eyes of recipients were collected, dissected, and digested in RPMI 1640 medium containing 10% FCS, 1 mg/ml collagenase D (Roche), and deoxyribonuclease I (Sigma-Aldrich) for 2 h. Following digestion, ocular cells were mechanically dissociated and filtered. The single ocular cell suspensions were used for flow cytometric analysis.

In certain experiments, as indicated, eye-infiltrating cells were collected by physical measures. Collected eyes were incised in cold PBS, followed by thorough flushing, to flush out the infiltrating inflammatory cells trapped in the eye cavities and tissues. The collected cells were analyzed by flow cytometry.

At the peak of inflammation induced by Th1 or Th17 cells, on day 5 postcell transfer, the total number of cells, as determined by flow cytometry or actual microsopical counting, was 2–3 million cells per eye. Of this total number, 250,000–400,000 were located on the flow cytometric spectrum at the area equivalent to that of live splenic lymphoid cells. Eight to 10% of these cells stained for CD4.
gene expression assay (Applied Biosystems). Recipient mice used for these assays were perfused with PBS from the left ventricle, to remove the blood from the eye.

**Histological analysis of recipient eyes**

Recipient eyes were collected at different time points postcell transfer, as indicated, and processed for histological analysis by H&E staining, using conventional methods (26).

**Results**

**Th1 divide faster than Th17 in the recipient spleen**

To monitor the pace of division by transferred Th1 and Th17 cells in the recipient mouse, we prepared highly purified populations of polarized cells of these two lineages derived from the same population of naive HEL-specific TCR Tg T cells (Fig. 1A). These cells were then labeled with CFSE and transferred into animals expressing HEL in the eye under control of the α-crystallin promoter (32). The proliferative status of these cells was then analyzed by flow cytometry of the dye intensity of cells accumulating in the recipient spleen. We and others have shown that adoptively transferred reactivated Th cells accumulate in the recipient spleen, where they undergo intense proliferation, before migrating to target organs (34, 35). In addition to the HEL transgenically present in the recipient eye, this Ag is most likely transferred with these activated cells as well. Data of a representative experiment are shown in Fig. 1B; similar results were obtained in two other experiments. Low levels of cell division are seen as soon as day 1, with Th1 cells showing higher proliferative activity than Th17. The faster division pace of Th1 became considerably more apparent on day 2, when the rate of division accelerated. By day 3, however, both Th1 and Th17 divided to an extent that was difficult to analyze due to further dilution of the CFSE (data not shown).

**Profound differences between Th1- and Th17-mediated inflammatory processes in their onset and duration**

The faster division rate of Th1 cells suggested that the inflammatory process mediated by these cells may outpace that mediated by Th17 cells. To examine this notion, we collected recipient mouse eyes at different time points after cell transfer and analyzed them for histological changes. Sections of representative eyes are shown in Fig. 2. Very similar changes were observed in at least five additional mice, examined at each time point, in at least three different experiments.

No changes were detected in eyes examined on days 1 or 2 postcell transfer (data not shown). The first changes were noted in recipients of Th1, on day 3, and included infiltration of inflammatory cells at the limbus and optic nerve head, the two sites of entry for inflammatory cells in this experimental model (36). In contrast, no changes could be detected in eyes of Th17 recipients on day 3. On day 4, the inflammation in Th1 recipient eyes already reached a high level of severity, characterized by proteinaceous and cellular exudates in both the anterior chamber and vitreous, as well as heavy infiltration of the retina. The first inflammatory changes in Th17 recipient eyes were detected on day 4 and closely resembled those seen in Th1 recipient eyes on day 3. Both Th1 and Th17 recipient eyes were severely inflamed on day 5 postcell transfer. Importantly, the inflammation in Th1 recipients subsided considerably on day 10 postcell transfer, whereas Th17 recipient eyes still retained a high level of the pathological changes. On day 15, very few inflammatory cells were seen in Th1 recipient eyes, whereas Th17 recipient eyes were still clearly inflamed. Additional experiments, not shown in this study, revealed that the ocular inflammation in Th17 recipients receded gradually at later time points, 20 and 25 days postcell injection, and only remnants of the inflammatory process could be detected on day 35. Thus, the more rapid onset of ocular inflammation in recipients of Th1-polarized cells correlates with the more rapid initial proliferation of these cells, but Th1-mediated inflammation terminates relatively quickly, whereas Th17-mediated inflammation persists for considerably more extended time period.

**Sustained ocular inflammation mediated by Th17 cells correlates with greater numbers of these cells in the target organ**

The sustained ocular pathology mediated by Th17 cells (Fig. 2) suggested that these cells may persist for longer times in the recipient mouse eyes, or alternatively, they may simply be more efficient at initiating sustained pathology. To investigate these questions, we isolated infiltrating cells from recipient eyes at different time points postcell transfer and quantified their numbers and origin by flow cytometry. In addition to Ag-specific T cells, non-Ag-specific bystander T cells are also recruited to the inflammatory site (37–39). To distinguish HEL-reactive T cells from other T cells, we used the anti-clonotypic mAb 1G12, specific to the donor (3A9) TCR (39, 40).

Fig. 3A depicts representative flow cytometric analyses of infiltrating CD4+ cells collected from eyes of Th1 and Th17 recipients at five time points postcell injection. In accord with the histological data (Fig. 2), T cell infiltration into the eye of Th1 recipients was detected first on day 3, whereas in Th17 recipients, it was first seen 1 day later, on day 4. A striking difference was observed between the two groups of recipients in the ratio between donor and host CD4+ cells at the different time points. Whereas donor (1G12+) cells comprised the majority of ocular CD4+ T cells at all tested time points in Th17 recipients, the percentage of donor Th1 cells...
declined dramatically after day 5, constituting the minority of infiltrating CD4$^+$ T cells on days 10 and 15. These differences were confirmed when we compared the absolute number of donor and host cells at the different time points (Fig. 3B). The numbers of donor Th1 cells in the eye rose faster than donor Th17 cells, but their number declined rapidly after reaching a peak on day 5. In contrast, the number of Th17 cells in the eye actually increased slightly between days 5 and 15. Counting inflammatory cells in eyes of Th17 recipients at later time points produced data in line with the histological observations mentioned above, showing a gradual reduction of the inflammatory process in these mice. Thus, the number of donor cells (CD4$^+$ 1G12$^-$) decreased from their peak of more than $3 \times 10^4$ per eye on day 15 to less than $2 \times 10^4$ on day 20, to $\sim 0.5 \times 10^4$ on day 25, and to less than $0.1 \times 10^4$ on day 35 postcell transfer. Unlike the profound differences between Th1 and Th17 donor cells, only small differences were noted between the number of host CD4 (1G12$^-$) cells in Th1 and Th17 recipient eyes (Fig. 3B).

Unlike Th1, Th17 cells are highly resistant to restimulation-induced cell death (RICD)

The sustained high number of Th17 cells in recipient eyes strongly suggested that these cells have enhanced survival in the setting of chronic antigenic stimulation. Elimination of CD4$^+$ T cells by chronically expressed self-Ags is known to be mediated by TCR-induced apoptosis, a process termed RICD (41). We therefore hypothesized that the difference in kinetics of the inflammatory process mediated by Th1 and Th17 is related to a difference in their susceptibility to RICD.

To compare the susceptibility of Th1 and Th17 cells to RICD, we prepared highly enriched Th1 and Th17 cells by the same methods used in the T cell transfer experiments and then induced RICD through the TCR, using anti-CD3 Ab. As shown in Fig. 4A, Th17 cells were highly resistant to TCR-induced apoptosis over a wide range of concentrations of anti-CD3 Ab, whereas Th1 cells were highly sensitive. To determine whether this resistance stemmed from a failure of Fas-induced apoptosis, we stimulated these cells with cross-linked anti-Fas mAb (Fig. 4B). As with TCR-induced apoptosis, Th17 cells were relatively resistant to Fas-induced apoptosis as compared with Th1 cells. This was not due to a difference in surface Fas levels; as shown by flow cytometry in Fig. 4C, Th17 actually had even slightly higher levels of surface Fas than Th1 cells.

The defect in apoptosis in response to anti-Fas Ab in Th17 cells was not as severe as for TCR-induced apoptosis, so we hypothesized that Th17 cells may also have impaired production of FasL, the physiological apoptosis-inducing ligand for Fas. Indeed, when populations of purified Th1 and Th17 cells were incubated with anti-CD3 Ab, Th17 cells produced dramatically lower levels of FasL protein (Fig. 4D). This effect most likely results from differences at the transcriptional level, because the baseline and induced FasL mRNA was present in much lower amounts in Th17 cells than in Th1 cells (Fig. 4E).

These observations suggest that the sustained presence of HEL-specific Th17 cells in the inflamed eye in the presence of chronically expressed Ag is due at least in part to the high resistance of these cells to RICD, following their exposure to the target Ag, HEL, which is abundantly expressed in the recipient eye. Resistance of Th17 to RICD is mediated both by reduced production of FasL and a cell-intrinsic resistance to apoptosis induced by exogenous FasL.

Low levels of FasL in Th17 recipient eyes

FasL is also expressed by non-T cells, including macrophages (42, 43) and tissue-resident cells, in particular in immune-privileged organs, such as the eye (44–46). To determine whether the expression of FasL in the eye differs between recipients of Th1 vs Th17 cells, we measured FasL transcript levels in the eyes of recipient mice of Th1 or Th17 cells. Data of four experiments are summarized in Fig. 5. Low levels of FasL transcript were detected in naive control eyes, and notably, higher levels were found in inflamed eyes. The level of FasL transcript in Th1 recipient eyes was higher than that seen in Th17 recipients, although expression was massively induced by transfer of both cell lineages. We assume that FasL transcript in recipient eyes is expressed by at least three cell types, i.e., resident ocular cells, infiltrating donor cells, as well as other inflammatory cells recruited into the inflammatory site, in particular macrophages. These data suggest that an additional layer of regulation at the level of FasL in recipient cells in the eye may also contribute to enhanced survival of Th17 cells.
**FIGURE 3.** Profound differences between Th1 and Th17 cells in their kinetics of target organ invasion. Five million polarized and reactivated Th1 or Th17 were injected into HEL-Tg recipients, and eyes were collected at the indicated time points. Eye-infiltrating cells were obtained, as described in Materials and Methods, and analyzed by flow cytometry. A. A representative experiment staining for CD4 and 1G12, a clonotypic marker for 3A9 TCR, used to identify donor (1G12+) and host (1G12-) CD4 cells. Th1 cells were first detected on day 3, reached a peak at ~day 5, and declined rapidly thereafter, becoming a minority among infiltrating cells by day 10. In contrast, Th17 were first detected on day 4, increased slowly in number, and remained the majority throughout the tested time period. B. Actual cell number per eye of donor and host CD4 cells at the different time points; means ± SEM of three independent experiments.

**Th17 cells survive in the inflamed eye better than Th1 cells**

To determine whether the resistance to RICD that we saw in Th17 cells in vitro correlates with enhanced survival of these cells in vivo, we sought to measure the viability of transferred T cells directly ex vivo. It was also formally possible that the enhanced numbers of Th17 T cells in vivo could be due to increased in vivo proliferation. To test this possibility, we labeled proliferating cells in recipients of Th1 or Th17 HEL-specific T cells with a short pulse of BrdU. As shown in Fig. 6A, similar levels of BrdU incorporation could be seen in Th1 and Th17 cells, indicating that the reason for reduced cell numbers of Th1 cells is not reduced in vivo proliferation. We then used staining with annexin V and 7-AAD to distinguish apoptotic cells in preparations of cells from the recipient eyes collected 5 days posttransfer (Fig. 6B). The cells from these eyes were collected by physical measures, to avoid possible collagenase effect. There was clearly a reduced level of viability among donor (1G12+) Th1 cells compared with Th17 cells, with this difference being underscored by the similarity between the viability levels among the recipient (1G12-) cells in Th1 and Th17 recipient eyes.

**Similarities and differences between non-CD4 cell populations recruited into Th1 or Th17 inflammation**

The populations of immune cells recruited into sites of inflammation include a variety of cell types. Because of their different cytokine secretion patterns, Th1 and Th17 cells are expected to recruit different subsets of these cells to sites of inflammation, which may change the outcome of T cell-mediated responses by these two subsets. To determine whether this may be the case, we collected cell infiltrates from recipient eyes and analyzed them by flow cytometry, using a battery of Ab known to identify major populations of immune cells. Flow cytometric data of representative analyses of cells collected on days 5 and 10 postcell injection are recorded in Fig. 7A, whereas actual numbers of cells stained with the different Abs, at five time points, collected in two or three experiments, are summarized in Fig. 7B. As shown in Fig. 7A, only negligible numbers of B cells (CD19+) were detected in recipient eyes and, therefore, data with these cells are not included in Fig. 7B.

In line with the more rapid onset of ocular inflammation in recipients of Th1, the accumulation of all tested cell populations was first detected on day 3 in recipient eyes of Th1, but on day 4 in Th17 recipients. As might be expected from the known ability of IL-17 to induce granulocyte chemotaxis (47, 48), infiltrates triggered by Th17 contained more than twice as many granulocytes (CD11b+Gr-1-) than infiltrates from Th1 recipients. By contrast, more CD8 cells were recruited into Th1 than into Th17 recipient eyes. Similar numbers of macrophages (CD11b+F4/80+) and dendritic cells (CD11c+F4/80-) were recruited into Th1 and Th17 recipient eyes, although dendritic cells accumulated more at later time points in Th17-mediated inflammation. These data indicate that the inflammatory processes induced in the eye by Th1 or Th17 cells lead to recruitment of partially different profiles of inflammatory cell populations to the site of inflammation in this model of autoreactive T cell-initiated tissue destruction.

**Discussion**

These findings show that the inflammatory response orchestrated by Th1 and Th17 cells directed against an identical eye-expressed Ag has remarkable differences in kinetics of inflammation and recruited cell types, which correlate with prolonged survival of Th17 cells at the site of inflammation.
Pathogenic Th17 were generated in our study in the presence of IL-6 and TGF-β. This observation seems to be different from that of McGeachy et al. (49), in which polarization by IL-6/TGF-β generates nonpathogenic Th17 cells, even when IL-23 was added. This difference could be attributed to major differences in the culture systems, as follows: 1) naive CD4 were used in our system, whereas T cells from immunized mice were used by McGeachy et al. (49); 2) IL-1, a cytokine known to promote Th17 pathogenicity (50–52), was added to our cultures, but not to those of McGeachy et al. (49); and 3) the use of different APC in the two studies, namely, irradiated naive spleen cells added to our system and the resident APC in the draining lymph nodes from immunized mice, were used by McGeachy et al. (49). Recent publications indicate that APC from immunized mice acquire the capacity to promote development of Treg cells (53–55) and, consequently, the overall propathogenic effect of these APC is conceivably lower than that of naive ones. The pathogenic phenotype of Th17 cells generated in our APC-stimulated cultures was further demonstrated by their high production of IL-22 and their profile of chemokine transcripts being very similar to that of pathogenic Th17 generated in the presence of IL-23 in the study of McGeachy et al. (49) (our unpublished data). It is also of note that nonpathogenic Th17 cells were also generated in our system in cultures polarized by IL-6/TGF-β, when the activation of naive CD4 cells was provided by anti-CD3 Ab (our unpublished data).

Polarized and reactivated Th1 and Th17 were compared in this study for their rate of division in the recipient spleen. Unlike naive CD4 cells, which mainly home to lymph nodes (56), adoptively transferred activated Th cells migrate mainly to the recipient spleen, where they multiply rapidly before moving on to seek their target Ag (34, 35). We measured the rate of cell division by CFSE dilution and found that Th1 divided substantially faster than Th17 cells at this phase. These differences between Th1 and Th17 cells were particularly clear on day 2 postcell transfer. Low levels of division were detected on day 1, whereas the intensive division of the transferred activated cells made it impossible to dissect the process at later time points.

In line with their faster rate of division, Th1 were also faster than Th17 in their invasion of the recipient mouse eye and initiation of the inflammatory process. Thus, cellular invasion was detected in eyes of Th1 recipients on day 3 postcell transfer, whereas in Th17 recipient eyes, this process was detected 1 day later, on day 4. It is noteworthy that the rate of eye invasion by the two
cell populations could also be affected by the differences in surface molecules such as the α4β7 integrin or CCR6, which we have previously reported to be elevated on Th17 cells invading the eye (26).

Even more striking than these changes in the onset of inflammation was the difference between Th1 and Th17 at the later phases of the pathogenic process. Th1 cell numbers in the affected eyes reached their peak by day 5 and declined rapidly thereafter, to approximately one-quarter of their peak number by day 10 and to less than one-tenth by day 15 postcell transfer. Th17 cells also reached peak levels in the recipient eye by day 5 in recipient transfer. Th17 cells also reached peak levels in the recipient eye by day 5 in recipient transfer, but in contrast to Th1, their proportion remained high and even slightly increased throughout the tested time period of 15 days (Fig. 3B). These differences between the transferred Th1 and Th17 were further highlighted by the relative similarity between the kinetics of host CD4 cells recruited by the inflammatory process in recipient eyes of Th1 or Th17 cells (Fig. 3B).

The persistence of Th17 cells in the presence of chronically expressed cognate Ag is most likely due to the remarkable resistance of these cells to apoptosis induced by restimulation through the TCR that we have demonstrated in this study. This resistance was accounted for by multiple failures in the Fas/FasL pathway in Th17 cells: both up-regulation of FasL and cell death triggered by Fas were impaired in Th17 cells, as compared with Th1 cells. FasL expression by restimulated T cells and in total RNA isolated from recipient eyes was also much higher in recipients of Th17 T cells.

The inflammatory processes in Th1 and Th17 recipient eyes also differed in the profile of recruited non-CD4 lymphoid cells (Fig. 7). Of particular interest is the remarkably larger proportion and actual number of neutrophils (CD11b^+Gr-1^+) in Th17 than in Th1 recipient eyes. This finding is in accord with observations in mice in which EAE was induced by Th1 or Th17 cells (23) and with reports of IL-17 acting as a stimulator of neutrophil mobilization (47, 48), as well as granulopoiesis (8). In contrast, more CD8 cells were recruited into Th1 than Th17 recipient eyes, in particular at the peak of the inflammatory process. Whether these CD8^+ T cells are responsible for some of the elimination of anti-HEL CD4^+ T cells.

**FIGURE 6.** In vivo proliferation and survival of transferred Th1 vs Th17 HEL-specific T cells. A. Recipients of Th1 or Th17 HEL-specific T cells were injected with BrdU, and CD4^+ T cells were analyzed for BrdU incorporation, as described in Materials and Methods. Data are representative of two experiments. The columns in the right panel summarize the mean percentage ± SEM of cells incorporating BrdU among the CD4^+ 1G12^+ cells in two separate experiments. B. Viability of T cells isolated from the recipient eyes was assayed with annexin V/7-AAD staining. The cells were collected by physical measures, as described in Materials and Methods. The columns in the right panels summarize the mean percentage ± SEM of live cells (annexin V/7-AAD negative) among CD4^+ 1G12^+ and CD4^+ 1G12^- populations in Th1 or Th17 cells, in two separate experiments. *p < 0.05.
is not known, but may be an interesting topic for future study. Unlike these differences between the two recipient groups, similar proportions and numbers of macrophages (CD11b^+ F4/80^+) were recruited into eyes of Th1 and Th17 recipients. Recruitment of dendritic cells (CD11c^+ F4/80^-) differed in its kinetics in the two groups, but was similar in proportion and cell numbers at the peak of inflammation.

Our data on the kinetics of inflammation induced by Th1 or Th17 shed new light on the controversial issue concerning the involvement of these two cell lineages at different phases of immune-mediated inflammation. Data accumulated in recent years suggest that both Th1 and Th17 may participate in early as well as late phases of experimental immune-mediated inflammatory processes. We show in this study for the first time that adoptively transferred polarized Th1 divide faster and invade the target organ considerably earlier than Th17. We also show that Th1 numbers decline rapidly in the inflamed eye, due mainly to apoptosis, whereas Th17 remain at site of inflammation for considerably longer. Our finding of Th17-induced inflammatory processes exhibiting slower kinetics and being sustained much longer than those of Th1 is thus in line with the data of Yoshimura et al. (28) and Hoyer et al. (29), who reported that Th17 are mainly involved at the later phase of the pathogenic autoimmune process. Yet, it is conceivable that during the complex process of pathogenic autoimmune immunity, Th1 and Th17 populations carry out different functions, at various phases of this process. Our findings with Th17 cells are also in line with the observations of Th17 being a major cellular component of chronic diseases in humans (10–21). We and others have recently shown that a portion of Th17 cells switches phenotype in vitro and expresses both IL-17 and IFN-γ.
or only IFN-γ (24, 64, 65). We further showed that the process of phenotype switching takes place in vivo as well, in Th17 recipient eyes (64). No information is available concerning the susceptibility to RICD of IL-17IFN-γ-double-positive cells, but it is conceivable that cells that acquire the Th1 phenotype become also more susceptible to RICD and undergo apoptosis at a rate much higher than the Th17 cells that retain their phenotype.

In summary, polarized TCR-Tg Th1 and Th17 differ remarkably in their mode of inflammation induction. Differences between the two lineages were noted in their rate of division, kinetics of target organ invasion, sustenance of the inflammatory process, susceptibility to RICD, and the populations of cells recruited into the affected eye. These observations are relevant to our understanding of the different roles played by Th1 and Th17 in immune-mediated inflammation. In particular, the sustained Th17-mediated inflammation that we have seen in our model is in accord with observations suggesting that Th17 cells play a major role in chronic conditions such as rheumatoid arthritis, multiple sclerosis, Crohn’s disease, psoriasis, and uveitis. The relative resistance to RICD of these cells may be beneficial, however, for long-lasting Th1 response against pathogens.

Acknowledgments
We thank R. Steven Lee for tail DNA analysis; the National Eye Institute for funding; the Jules Stein Eye Institute and the Wilmer Eye Institute for the use of their facilities; and C. C. Chan, J. A. DeMartino, J. M. Farber, and I. Gery for helpful discussions. Acknowledgment of receipt of NIH grant funds for studies reported herein does not imply approval by the Department of Health and Human Services or the U.S. Public Health Service.

Disclosures
The authors have no financial conflict of interest.

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Th17 CELLS INDUCE SUSTAINED INFLAMMATION


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