Active Participation of Antigen-Nonspecific Lymphoid Cells in Immune-Mediated Inflammation[1]

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The pathogenic process of tissue-specific autoimmune disease depends to a large extent on recruitment of Ag-nonspecific cells into the target tissue. Little is known, however, about the recruitment process and the features that characterize the recruited cells. In this study, we analyzed the recruitment of Ag-nonspecific lymphoid cells into an inflammatory site by using an experimental system in which TCR-transgenic Th1 cells are adoptively transferred to induce ocular inflammation in recipient mice that express the target Ag in their eyes. A sharp increase in number of all host cell populations was observed in the recipient spleen, reaching a peak on day 4 postcell transfer and declining thereafter. A large portion of the host's spleen CD4 cells underwent phenotypic changes that facilitate their migration into the target organ, the eye. These changes included increased expression of the chemokine receptor CXCR3, and the adhesion molecule CD49d, as well as a decline in expression of CD62L. The host lymphocytes migrated into the recipient mouse eye more slowly than the donor cells, but became the great majority of the infiltrating cells at the peak of inflammation on day 7 postcell injection. Interestingly, the mass migration of host T cells was preceded by an influx of host dendritic cells, that reached their peak on day 4 postcell injection. The eye-infiltrating host CD4 lymphocytes underwent additional changes, acquiring a profile of activated lymphocytes, i.e., up-regulation of CD25 and CD69. Our results thus provide new information about the active participation of Ag-nonspecific lymphoid cells in immune-mediated inflammation. *The Journal of Immunology,* **2006, 177: 3362–3368.**

Tissue-specific autoimmune diseases are dependent on amplification mechanisms involving invasion of Ag-specific T cells and the ensuing recruitment of Ag-nonspecific cells into the target tissue, where the Ag is located (1, 2). This pathogenic process has been studied mainly in experimental systems in which diseases such as experimental autoimmune encephalitis (EAE),[3] type-1 diabetes, or experimental autoimmune uveoretinitis (EAU) are induced by adoptively transferred lymphocytes with specificity toward Ags located in the target tissue (1, 3–7). Little is known, however, about the actual magnitude of the recruitment process and the identity and features of the Ag-nonspecific cells that are recruited into the target tissue.

The migratory behavior of lymphoid cells is determined by several molecules expressed on their surface, including chemokine receptors and adhesion molecules. A chemokine receptor that plays a major role in the migration of inflammation-inducing Th1 cells is CXCR3 (8–10). Two major adhesion molecules, which affect migration, are CD49d (VLA-4) and CD62L (L-selectin), with the former being up-regulated and the latter undergoing down-regulation in effector lymphocytes that migrate into nonlymphoid tissues (8, 11–14).

We have investigated the cells and molecules that participate in immune-mediated inflammation by using an experimental model in which transgenic (Tg) mice expressing hen egg lysozyme (HEL) in their eyes develop ocular inflammation following adoptive transfer of TCR Tg HEL-specific Th1 cells (15, 16). In a previous study (8), we characterized the features of the donor Th1 cells and the remarkable changes in their surface markers during the various stages of their activation in vitro, early migration to lymphoid tissues, and invasion of the target eye. In the present study, we monitored the participation of host cells in the different stages of the inflammatory process. The availability of a clonotypic Ab specific to the TCR of the donor T cells made it possible to differentiate between donor and host cells in the target eyes and secondary lymphoid organs.

We found that the injected donor Th1 cells triggered a sharp increase in the recipient's spleen size and cellularity, with the participation of various populations of spleen cells. Similar to donor cells, a significant portion of the host spleen $CD4^+$ T cells was found to undergo changes in their surface markers, which facilitate the migration into nonlymphoid tissues (8). Unlike the rapid influx of donor Th1 cells into the affected eyes, host cells accumulated more slowly, yet eventually became the majority cell population. Different host lymphoid cell populations were identified in the affected eye, including a surprisingly large number of CD11c dendritic cells (DCs). Interestingly, the surface marker profile of the eye-infiltrating CD4 host cells differed from that of the corresponding populations located in the spleen or blood by exhibiting a profile shift toward that of activated T cells.

Materials and Methods

Mice

HEL-Tg mice, expressing membrane-bound HEL under control of the αA-crystallin promoter, on the FVB/N background, were generated as described previously (17). HEL-specific TCR Tg mice, on the B10.BR background, designated "3A9" (18), were a gift from M. Davis (Stanford University, Stanford, CA). Tg mice from each of the two lines were mated to produce (FVB/N × B10.BR)F_1 hybrids, expressing either HEL in their

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalitis; EAU, experimental autoimmune uveoretinitis; Tg, transgenic; HEL, hen egg lysozyme; DC, dendritic cell; FCM, flow cytometric; ALPC, allophycocyanin; SA, streptavidin.

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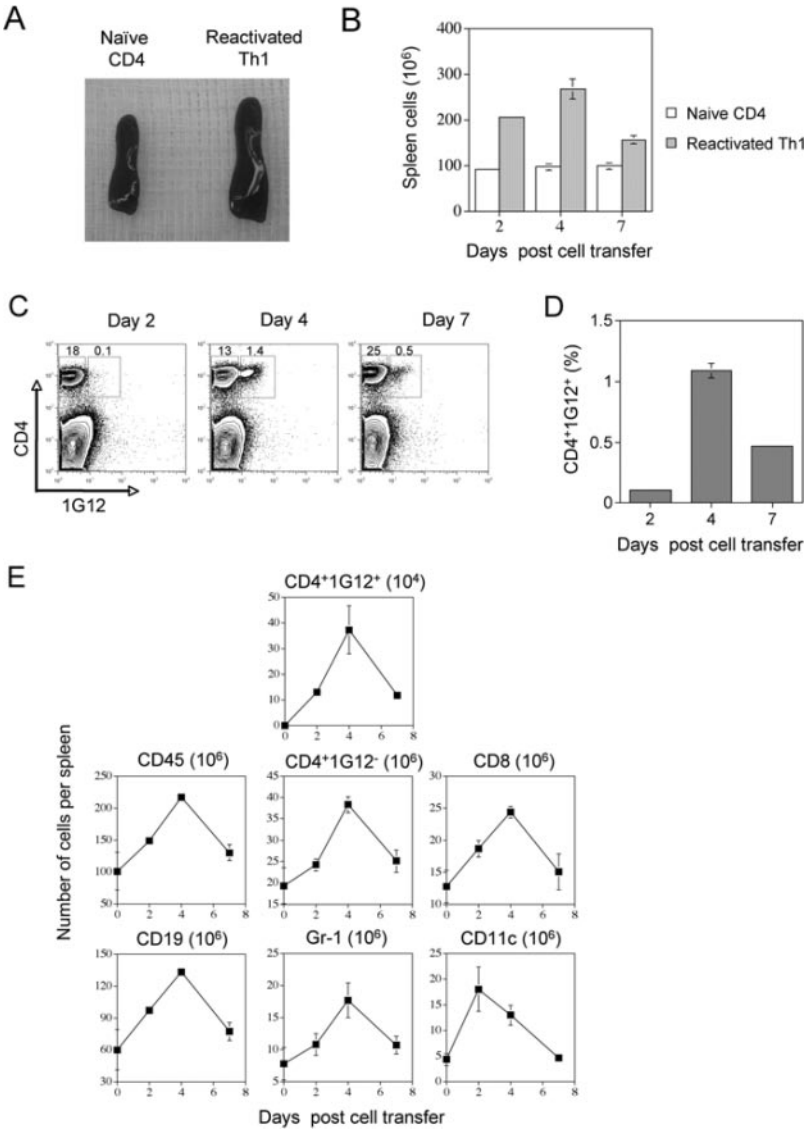

FIGURE 1. Adoptively transferred reactivated Th1 3A9 cells induce increases in size and cellularity of the recipient spleen. *A*, Recipient spleens collected 4 days following injection with 2×10^6 reactivated Th1 or naive CD4 3A9 cells. *B*, Number of nucleated cells in the spleen of recipient mice, injected with 2×10^6 naive or reactivated CD4 3A9 cells, at the indicated time points. The bars represent means ± SEM of three separate experiments. *C,* FCM analysis of donor and host CD4 cells ($1G12^+$ and $1G12^-$, respectively) in spleens of recipients at different time points following injection with 10×10^6 reactivated Th1 cells from 3A9 donors. The recorded values represent percentages of host ($1G12^-$) or donor ($1G12^+$) cells in total spleen cells. *D*, Percentage of donor CD4 cells in total spleen cells at the indicated time points. The bars represent means ± SEM of three separate experiments. *E*, Spleen cells collected from recipient mice at different time points following injection with 2×10^6 reactivated Th1 3A9 cells were analyzed by FCM for their being of donor ($1G12^+$) or host origin ($1G12^-$) and for their expression of lymphoid cell population markers. The data are recorded as the calculated number of cells per spleen, and the values are means ± SEM of six mice per time point, obtained in two separate experiments. For all panels, the difference between the cell number on day 4 was significantly higher ($p < 0.05$) than those on days 0, 2, or 7.

eyes, or the 3A9 TCR on their T cells. The mice expressing HEL in their eyes are designated here "HEL-Tg," whereas those expressing 3A9 TCR are named 3A9. Only such F_1 hybrid mice of the two lines were used in the present study. In all adoptive transfer experiments the cells used were from 3A9 donors, whereas recipients were HEL-Tg mice. The mice were housed in a pathogen-free facility, and all manipulations were performed in compliance with the National Institutes of Health Resolution on the Use of Animals in Research.

Generation of HEL-specific Th1 lymphocytes

Th1 cells were prepared as described in detail elsewhere (15, 16). Briefly, purified CD4 cells (>95%) from 3A9 mice were incubated for 3 days with HEL (at 2 μg/ml) and irradiated (30 Gy) syngeneic wild-type naive spleen cells, serving as APC, in Th1 polarizing conditions (IL-12 and anti-IL-4 Ab (15, 16)). Following ensuing incubation with IL-2 for 4 days, polarized Th1 cells were stimulated again with HEL, APC, and the Th1 polarization mixture to produce the reactivated cell preparation, which is highly pathogenic (8). The Th1 cell polarization was qualified by assessment of intracellular cytokines and cytokine production by these cells, as detailed elsewhere (15); Th1 cell suspensions produced high levels of IFN-γ, but almost no IL-4, expressed strongly surface IL-18R, but little of the T1/ST2 marker.

Induction of ocular inflammation by adoptive transfer of HEL-specific Th1 lymphocytes

Reactivated Th1 cells (2×10^6, 5×10^6, or 10×10^6, as indicated) were injected in a volume of 0.2 ml via the tail vein into naive HEL-Tg mice to induce ocular inflammation. Recipient mice were sacrificed at different time points following cell transfer, as indicated. Blood, spleen, and eyes of recipient mice were collected and prepared for flow cytometric (FCM) analysis.

FCM analysis of surface marker expression

Abs were conjugated with FITC, PE, PerCP, or allophycocyanin (ALPC), as indicated. mAbs against CD4 (PE or ALPC), CD8 (PE), CD19 (PerCP), CD11c (ALPC), CD49d (PE), CD62L (PE), CD69 (PE), and CD25 (PE) were purchased from BD Pharmingen. Rabbit anti-CXCR3 Ab was obtained from Zymed Laboratories, whereas goat anti-rabbit IgG Ab (PE) was from Southern Biotechnology Associates. A clonotypic mAb specific for the Tg TCR of the 3A9 mice, designated 1G12, a gift from E. Unanue (Washington University, St. Louis, MO), was used conjugated with FITC. Control staining was performed using the following isotype Abs: rat IgG 2a, κ (ALPC), rat IgG1, λ (PE), rat IgG2b, κ (PE), mouse IgG1 (FITC), hamster IgG1, λ (PE) (all obtained from BD Pharmingen), and rabbit IgG (Pierce). Anti-CD16/CD32 (BD Pharmingen) was used to block FcRs in all the stainings.

Blood, spleen, and eyes of recipient mice were collected at different time points following adoptive transfer of Th1 cells. Single-cell suspensions of blood and spleen cells were prepared by conventional methods. Eyes were dissected and then digested in RPMI 1640 medium containing 10% FBS and 1 mg/ml collagenase (Sigma-Aldrich) for 2 h at 37°C. Collected cells were filtered through a cell strainer, followed by RBC lysis with ACK buffer and washing in FACS buffer. FCM analysis was performed on a FACSCalibur (BD Biosciences). One million events were acquired for cell samples recovered from spleen or blood of recipient mice, whereas all cells collected from recipient eyes (below 10^6 events) were analyzed.

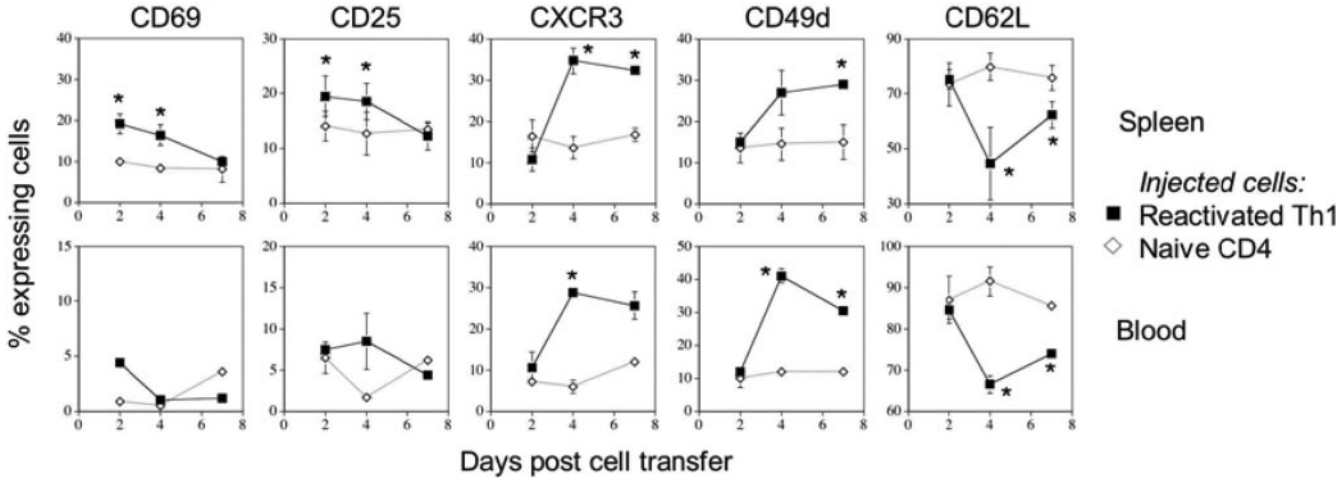

FIGURE 2. Changes in expression of surface markers on host spleen or blood CD4 cells at different time points following adoptive transfer of reactivated Th1 or naive CD4 cells from 3A9 donors. HEL-Tg recipient mice were injected with 10^7 donor cells, as indicated, and expression of surface cell markers on $CD4^+1G12^-$ cells was analyzed by FCM. The recorded data are means ± SEM of three separate experiments, with a total of nine mice tested for each time point (*, $p < 0.05$).

BrdU incorporation assay

HEL-Tg recipient mice of reactivated Th1 3A9 cells (5×10^6) were injected i.p., on day 6 postcell transfer, with 0.5 mg of BrdU (BD Pharmingen). Recipient mice were euthanized 4 h later, and the infiltrating cells in their eyes were collected and stained with PE-conjugated CD4 Ab and biotin-conjugated 1G12 Ab, plus streptavidin (SA)-APC (BD Pharmingen). Cells were then fixed and permeabilized using BD Cytofix/Cytoperm buffer (BrdU Flow Kit; BD Pharmingen), stored overnight, and then stained with FITC-conjugated anti-BrdU Ab (BD Pharmingen). Gated CD4 cells were then analyzed by FCM for BrdU incorporation.

Immunofluorscence staining of eye tissues

Eyes were removed from mice 4 days after T cell transfer and frozen in OCT-embedding medium (Sakura Finetek). Frozen sections (8-μm thick) were fixed in acetone at −20°C, and immunofluorescence was performed using the tyramide amplification method (TSA-Direct kit; Molecular Probe-Invitrogen Life Technologies). Frozen sections were blocked with blocking buffer (TSA-Direct kit), and endogenous peroxidase activity was quenched with peroxidase-quenching reagent (DakoCytomation) for 10 min at room temperature. A total of 0.5 μg/ml purified anti-CD11c (clone N418; eBioscience) and 0.5 μg/ml biotinylated anti-CD4 (clone RM4-5; eBioscience) was applied for 1 h at room temperature. Slides were washed and incubated with SA-HRP for 30 min. Biotinylated anti-CD4 was detected with Tyramide-Alexa Fluor 594 (TSA-Direct kit). Next, HRP activity was quenched with peroxidase-quenching reagent (DakoCytomation) for 10 min, and biotin sites blocked with excess unlabeled avidin and biotin (Avidin/Biotin blocking kit). The sections were then incubated with biotinylated-goat anti-hamster IgG (Jackson ImmunoResearch Laboratories). Slides were washed and incubated with SA-HRP conjugate (TSA-Direct kit). Staining by hamster anti-CD11c was visualized by amplification of the signal with Tyramide-Alexa Fluor 488 (TSA-Direct kit). Slides were mounted with Flouromount G (Southern Biotechnology Associates) and were analyzed by fluorescence microscopy (Axioplan 2; Carl Zeiss).

Statistical analysis

Student's *t* test was used to determine the significance of differences between the means of different groups.

Results

Profound expansion of host cells in recipients' spleen

Groups of HEL-Tg mice were injected with either naive CD4 or reactivated Th1 3A9 cells, and the recipients' spleens were collected at different time points. A pronounced increase in the spleen size of recipient mice injected with 2–10 $\times$ 10^6 reactivated Th1 cells was regularly observed (Fig. 1*A*). This phenomenon of increase in spleen size was similarly observed in wild-type recipients, indicating that HEL expression by the Tg recipient mouse does not participate in the biological process (data not shown). The increase in size began on day 2, reached a peak around day 4, and was followed by a gradual decline thereafter (Fig. 1*B*).

FCM analysis of spleens from recipients injected with reactivated Th1 cells revealed that the proportion of 3A9 donor cells, identified by the clonotypic Ab 1G12, increased rapidly in the recipient spleen, reaching a peak on day 4 and declining on day 7 (Figs. 1, *C–E*, *top panel*). The donor cells comprised, however, merely a negligible minority in the recipient spleens, reaching for example an absolute number of only ~400,000 at their peak on day 4 in the experiments presented in Fig. 1*E*, in which 2×10^6 reactivated Th1 cells were injected. FCM analysis on days 0, 2, 4, and 7 following cell transfer was also performed with Abs against different cell surface markers (Fig. 1*E*). This analysis showed that most nucleated cells collected from the recipient spleens expressed CD45. An increase in the absolute number of $CD45^+$ cells was seen in these spleens on day 2 postcell injection, reaching a peak of >200 million cells (approximately twice the number of cells in untreated mice) on day 4 and decreasing on day 7 (Fig. 1*E*). The other populations of host leukocytes measured at the different time points following injection of the reactivated 3A9 Th1 cells included CD4 and CD8 T cells, B cells (CD19), granulocytes (GR-1), and DCs (CD11c) (Fig. 1*E*). Four of the five populations exhibited similar increases in their peak number, to approximately double their numbers on day 0. The only population that had a larger increase in cell number was that of $CD11c^+$, which more than tripled in size. The $CD11c^+$ population also differed from the other populations in its kinetics, reaching a peak on day 2, whereas all other populations reached their peak on day 4.

In contrast to the observations in recipients of reactivated Th1 cells, no changes in the numbers of either donor or recipient cells were found in the spleen of recipients of naive 3A9 CD4 cells throughout the tested time period (Fig. 1*A* and data not shown).

Taken together, these results demonstrate an expansion of host lymphoid cells in the spleens of recipient mice injected with reactivated Th1 cells. This expansion is presumably triggered by the adoptively transferred reactivated lymphocytes.

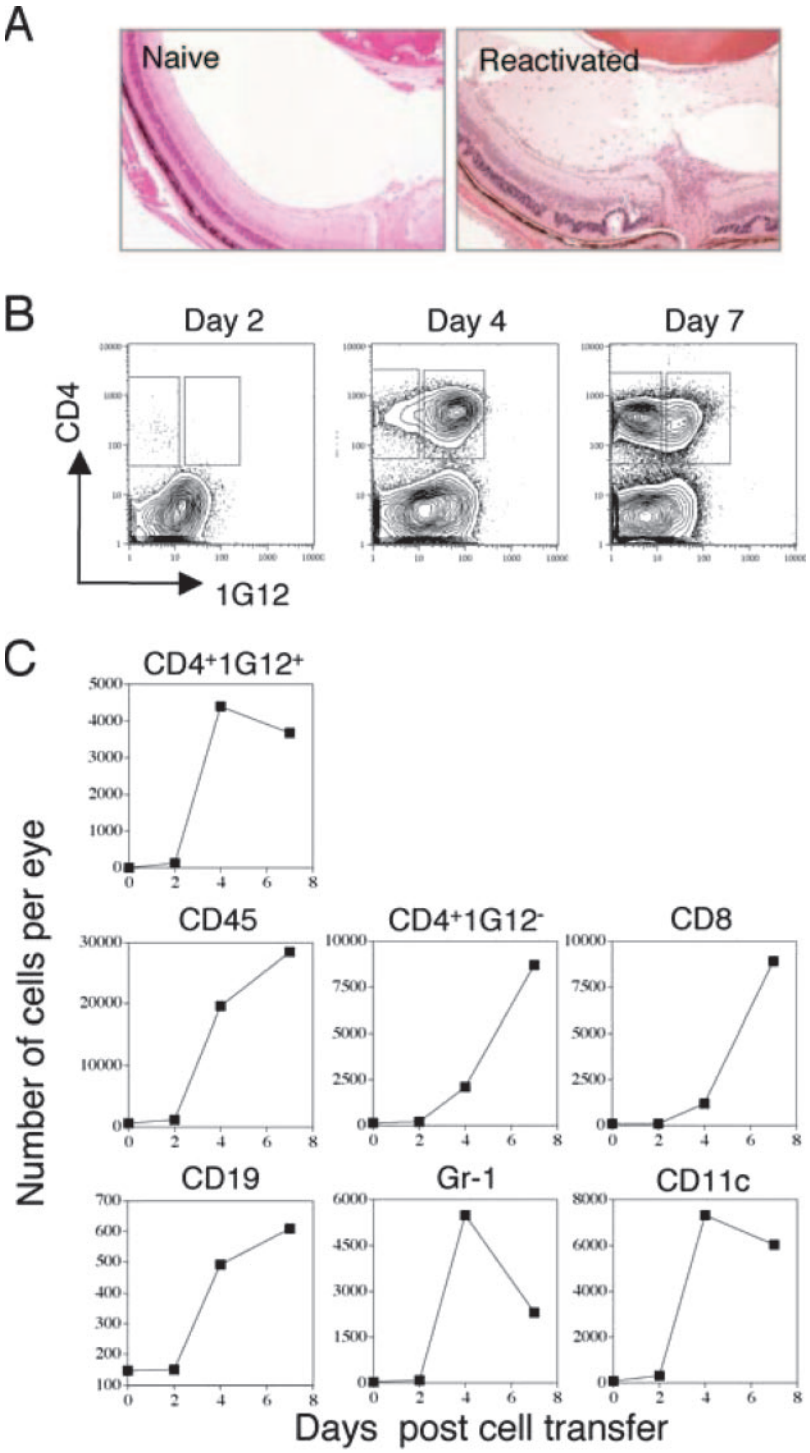

FIGURE 3. Identification of host cells that participate in the process of ocular inflammation. *A*, Typical changes in recipient mouse eye, 7 days following adoptive transfer of 2×10^6 naive CD4 cells, or reactivated Th1 3A9 cells, showing heavy cellular infiltration in the vitreous and retina (H&E; original magnification, ×10). *B*, Representative FCM analyses of the eye-infiltrating CD4 cells, showing the host cells ($1G12^-$) being the minority on day 4 postcell injection, but becoming the majority on day 7. *C*, Identification of host cell populations that infiltrate the inflamed recipient mouse eyes. Infiltrating cells were collected following collagenase treatment of recipient eyes on days 2, 4, and 7 postinjection of 2×10^6 reactivated Th1 3A9 cells and analyzed by FCM for population markers. Donor and host cells were analyzed separately by gating on $1G12^+$ or $1G12^-$. The recorded data were collected in a representative experiment in which cells collected from eyes of three mice were pooled and examined in each time point. Similar results were obtained in another experiment.

Phenotype changes on host $CD4^+$ lymphocytes in spleen and blood of recipient mice

To determine the expression of function-related surface markers on host $CD4^+$ lymphocytes in recipient mice, we conducted FCM analysis with Abs against two activation markers, CD25 and CD69 (19, 20), and three molecules that determine to a large extent the migratory properties of T lymphocytes, i.e., the chemokine receptor CXCR3 (8–10) and the adhesion molecules CD49d (VLA-4) (8, 11, 12) and CD62L (L-selectin) (8, 13, 14). Fig. 2 summarizes the data obtained in four experiments with spleen and blood cells of recipients of naive CD4 or reactivated Th1 cells from 3A9 donors. There was little change in the expression of any of the surface molecules on host $CD4^+$ cells from recipients of naive CD4 cells throughout the 7-day period of the experiments. In contrast, notable changes were observed on host spleen and blood $CD4^+$ cells of recipients of reactivated Th1 cells. The percentage of cells expressing CD25 and CD69 was moderately higher in recipients of the reactivated cells than in recipients of naive CD4 cells on days 2 and 4 postcell injection, but declined to the baseline level on day 7. The proportions of host CD4 cells expressing CXCR3 and CD49d in recipients of the reactivated Th1 cells were similar on day 2 to that of the control, i.e., ~10%, but these proportions increased sharply on day 4 to ~30–40%, with little or no additional change by day 7. Furthermore, the expression of

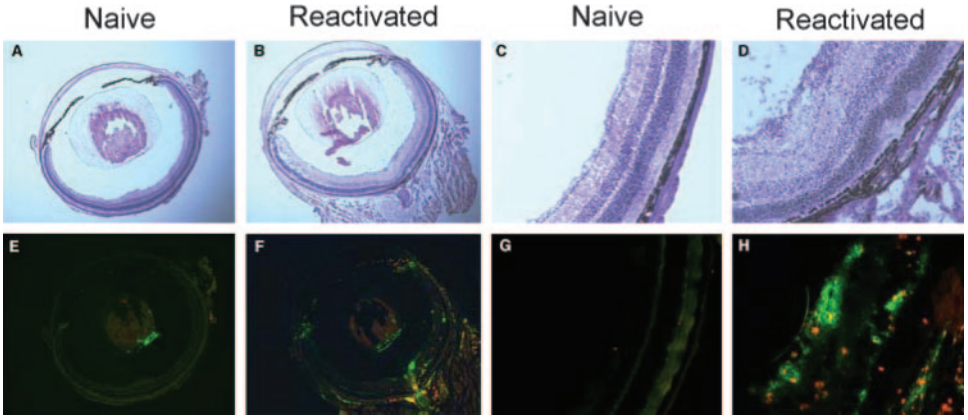

FIGURE 4. Immunofluorescence staining of recipient mouse eye for DCs and T cells. Eyes were taken from mice 4 days after transfer of naive CD4 cells (*A*, *C*, *E*, *G*) or reactivated 3A9 Th1 cells (*B*, *D*, *F*, *H*) and stained with H&E (*A–D*) or with Abs against CD11c (green) and CD4 (red) (*E–H*). Low-power views (×5) are presented in *A*, *B*, *E*, and *F*, and high-power magnification (×25) of representative portions of the retinas are presented in *C*, *D*, *G*, and *H*.

CD62L on these host cells declined from its baseline high level of 80–90% on day 2 to 50–70% on day 4, with a partial rebound on day 7.

These results suggest that a portion of the host $CD4^+$ T cells in recipients of reactivated Th1 cells underwent activation and modification in their surface markers to a profile of effector cells that preferentially migrate into nonlymphoid tissues (8–14).

Migration of host lymphoid cells into the recipients' eyes

Eyes of recipients of reactivated Th1 cells developed severe inflammatory reactions characterized by infiltration of lymphoid cells into multiple ocular tissues (Fig. 3*A*). To identify the infiltrating cells and to monitor the pattern of their migration, we extracted cells from affected eyes by collagenase treatment at different time points postcell transfer and identified them by FCM. Donor and host cells were identified by their positive or negative staining with the clonotypic 1G12 Ab, respectively, and the different lymphoid cell populations were identified by their specific cell markers. In line with our previous study (8), massive invasion of donor $CD4^+$ T cells ($1G12^+$) was observed on day 4 postcell injection, but these cells were outnumbered by host $CD4^+$ T cells ($1G12^-$) on day 7 (Fig. 3*B*).

FCM analysis of the cell infiltrate with population-specific Abs revealed the involvement of different lymphoid cell populations in the inflammatory process (Fig. 3*C*). Similar numbers of the host's CD4 and CD8 cells were found to invade the affected eyes, with the highest numbers of ~9,000 per eye recorded for each of these T cell populations on day 7. Host B cells (CD19) were also seen among the infiltrating cells, but at low numbers (~600/eye). In contrast, considerable numbers of granulocytes (GR-1) were recruited into the inflamed eye, with the peak of ~5,000/eye reached on day 4 and a steep decline thereafter.

Of particular interest is the participation of $CD11c^+$ DCs in the inflammatory process. By FCM, substantial numbers of $CD11c^+$ cells migrated rapidly into the affected eyes, reaching a peak of ~7,000 per eye on day 4 and declining slightly on day 7 (Fig. 3*C*). In addition, analysis by immunofluorescence staining of eye tissue at day 4 demonstrated the presence of $CD11c^+CD4^-$ DCs in eye tissues (Fig. 4). DCs were clustered with $CD4^+$ T cells in the retina, particularly in regions near the optic nerve, as well as at the limbus area, the two loci that are the entry points for the infiltrating cells in these eyes (15).

These results thus demonstrate the involvement of host lymphoid cells in the process of adoptively transferred ocular inflammation, and identify DCs as a primary component of the initial inflammatory infiltrate.

Phenotypic changes on the host $CD4^+$ lymphocytes infiltrating into the target eye

To examine the expression of function-related surface markers on tissue-infiltrating host CD4 lymphocytes, we analyzed by FCM the

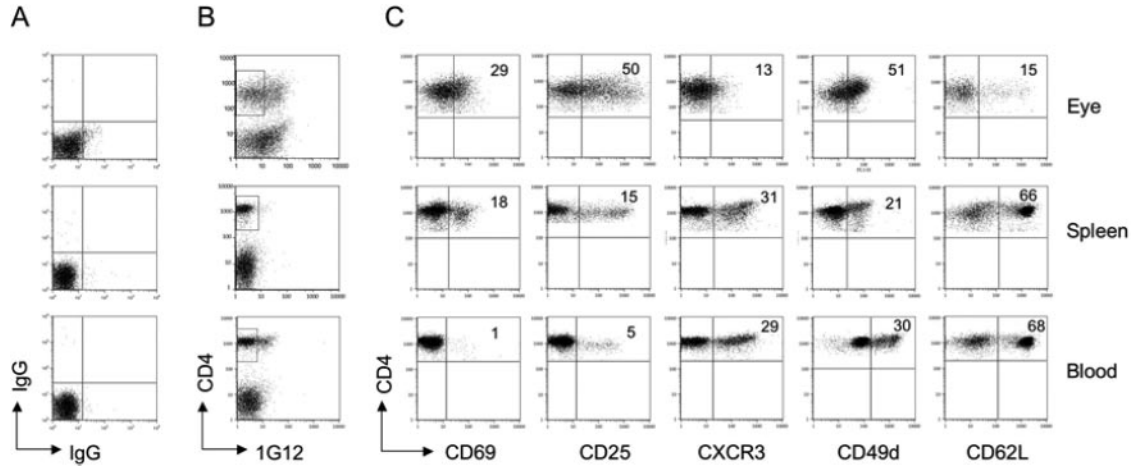

FIGURE 5. Profile of surface markers on host cells that infiltrate the recipient mouse eyes differs from that of host cells in the spleen or blood. Eyes, spleen, and blood samples were collected from recipient mice 7 days following adoptive transfer with 10^7 reactivated 3A9 Th1 cells. Single-cell suspensions of spleen, blood, and eye-infiltrating cells, collected by collagenase treatment, were stained with isotype controls (*A*), or were gated by FCM for the $CD4^+1G12^-$ (host) population (*B*) and analyzed for their expression of cell surface markers (*C*). The data shown are of a representative experiment in which cells of three mice were pooled for each organ. Similar results were obtained in two other experiments.

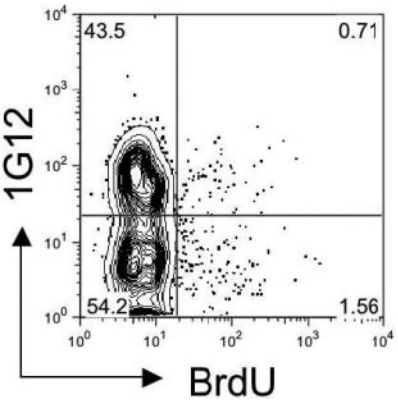

FIGURE 6. Proliferation of infiltrating donor and host cells in recipient mouse eyes. HEL-Tg mice injected with 5×10^6 reactivated Th1 cells from 3A9 donors were pulsed on day 6 with BrdU for 4 h, and infiltrating cells in eyes of three recipients were collected, pooled, and stained for CD4, 1G12, and BrdU, as detailed in *Materials and Methods*. Cells gated for CD4 were analyzed by FCM for 1G12 expression and BrdU incorporation.

$CD4^+1G12^-$ population collected from the inflamed eyes on day 7 postcell injection, as well as the corresponding cell population in the spleen and blood of the same recipient mice (Fig. 5). In accord with the data recorded above (Fig. 2), similar profiles are seen with the spleen and blood lymphocytes. In contrast, remarkable differences were found between cells from these lymphoid organs and the eye-infiltrating lymphocytes. Thus, the expression of the two activation markers, CD25 and CD69, as well as of the adhesion molecule CD49d was higher on host CD4 cells collected from inflamed eyes than on the corresponding cells from spleen or blood. In contrast, expression of the chemokine receptor CXCR3 and the adhesion molecule CD62L was lower on eye-infiltrating cells than on spleen or blood cells.

The change in expression of surface molecules on host CD4 cells following their invasion of the eye resembles the change we observed on the donor T cells after they migrated from the circulation into the eye (8). Similar changes are also seen on T cells following their reactivation in vitro (8). Taken together, these FCM data suggest that infiltrating host T cells undergo an activation process following their migration into the inflamed eye.

Cell proliferation in the inflamed eye

Lymphocyte activation may bring about cellular proliferation. To examine the proliferative activity of infiltrating cells in recipient mouse eyes we used the BrdU assay. Cells collected from inflamed eyes of recipient mice, on day 6 postcell transfer and after a 4-h pulse in vivo with BrdU, were analyzed by FCM for BrdU incorporation. As seen in Fig. 6, BrdU incorporation was found in small fractions of both host and donor cells, indicating proliferation in these positive cells.

Discussion

This study provides new information concerning the participation of recruited cells in the pathogenic process of T cell-mediated inflammation. The experimental system of ocular inflammation used here serves as a model for pathogenic immune response against tissue-specific self Ags, and we propose that the observations concerning the host cells made in this system could be applicable to other systems in which Ag-nonspecific lymphoid cells are recruited into immune-mediated inflammatory sites such as in EAE, EAU, or other autoimmune diseases. One primary observation in this study was the profound increase in number of spleen cells in mice receiving reactivated Th1 cells. Donor cells divided rapidly in these mice (8), but they comprised merely a small fraction (<1%) of the total nucleated cells in these enlarged spleens. Yet, we assume that the reactivated Th1 donor cells are responsible for the increase in number of spleen cells, because no such phenomenon was seen in the control recipients injected with naive CD4 cells. The mechanism(s) that drives this remarkable increase in spleen cell number is unknown and is currently under investigation, with preliminary data, suggesting that both cell proliferation and migration of lymphoid cells from other organs contribute to the increase in the spleen size. A similar increase in spleen size was also observed in wild-type hosts injected with reactivated Th1 3A9 cells, indicating that the phenomenon is unrelated to HEL expression in the Tg recipient mice. With the exception of the DC ($CD11c^+$) population, all other host cell populations reached their peak number on day 4, when they approximately doubled in size (Fig. 1). The DC population, in contrast, reached its peak on day 2, with an increase in size of >3-fold. DCs have been shown to direct lymphocyte migration to different organs (21–23), and their early increase in number in the recipient spleens suggests that they played a role in recruiting cells from other lymphoid populations to these spleens.

It is possible that minute amounts of HEL are carried over into the recipient mice along with the transferred Th1 cells, but apparently they have no effect on the immune response of the recipient mice. In experiments not shown here, naive wild-type CD4 cells were cultured with HEL, using the same procedure used for reactivation of Th1 cells. Injection of 5×10^6 of these wild-type CD4 cells into HEL-Tg mice did not induce any increase in spleen size or ocular inflammation. Furthermore, no immune response against HEL could be detected in the recipient mice by the lymphocyte proliferation or Ab assays. Moreover, these negative results are in line with a previous study (17) in which we showed that HEL Tg mice develop immunotolerance to HEL, apparently due to HEL expression in thymi of these mice. Another finding of interest concerns the effect of the transferred Th1 cells on the profile of surface molecules on host spleen CD4 lymphocytes (Fig. 2); to the best of our knowledge, the present study is the first to record such data. Particularly important is the up-regulation of CXCR3 and CD49d, as well as the down-regulation of CD62L on a large portion of these host cells. These combined changes in surface molecules are known to prepare T lymphocytes for migration into nonlymphoid tissues (8–14), and it is conceivable that many of the host cells acquiring the new phenotype are those that migrate from the spleen into inflammation sites, the eye in this study. Indeed, the profile of these modified host cells is very similar to that of the donor CD4 cells before their migration from the recipient's spleen to their eyes (8). This notion about the migration of $CXCR3^+$ cells into inflamed tissues also agrees with our previous observation of high levels of CXCR3 ligands (monokine-induced by IFN-γ/CXCL9 and IFN-γ-inducible protein-10/CXCL10) in the target mouse eyes (16).

Using FCM analysis of the eye-infiltrating cells, we monitored quantitatively, for the first time, the participation of the major host cell populations in the inflammatory process in the affected eye (Fig. 3). The availability of the clonotypic Ab, 1G12, which identifies the donor cells, made it possible to differentiate between donor and host CD4 cells. Notably, the migration kinetics of host T lymphocytes differed from that of the donor cells. The massive migration of T lymphocytes into the eye consisted mainly of donor cells on day 4, but the number of these cells declined slightly on day 7, when the great majority of T cells were from the host (Fig. 3). The total number of infiltrating cells was also higher on day 7 than on day 4. Thus, our analysis of the cellular infiltrate provides quantitative support to the notion that at the peak of the inflammatory process, the majority of

lymphocytes at a tissue-localized inflammation are recruited cells, with no specificity toward the target Ag (1, 2).

Large numbers of DCs were found in the affected recipient eyes. It is possible that these cells were recruited from the peripheral blood by chemokines such as MIP3α/CCL20, released from nonimmune tissue cells, similar to the observations made with models of inflammatory bowel disease (24) or pulmonary inflammation (22, 25). DCs migrated to the target eye before the host B or T cells, reaching a peak on day 4 and declining slightly on day 7. The function of these DCs in the inflammation process is not yet clear; however, there are several possible implications of these findings. For example, DCs within the eye may present HEL to donor T cells, resulting in the activation of these T cells (8). In addition, DCs may be involved in recruitment of lymphocytes into the eye, by releasing chemokine ligands of CXCR3 or CCR5, such as CXCL9–11, or CCL3–5, which act to attract Th1 cells into tissues (26).

Another noteworthy observation of this study is the difference between the profiles of surface markers of the host CD4 cells in the spleen or blood and in the inflamed eyes (Fig. 5). Particularly interesting is the finding of lower expression levels of CXCR3 (13 vs 31%) and the higher levels of CD25 and CD69 on the host-infiltrating cells than on the corresponding spleen cells (50 and 29% vs 15 and 18%, respectively). This change in the surface marker profile on host CD4 cells is similar to, albeit less pronounced than, that we observed with the eye-infiltrating donor cells (8). In that study, the expression of CXCR3 declined from 88% on spleen cells to 11% on the eye-infiltrating cells, whereas the expression of CD25 and CD69 was 5 and 7% on spleen cells and 40 and 40% on infiltrating cells, respectively (8). The finding of a profile change on eye-infiltrating donor cells also resembles the observation with T cells that induce EAU (7), or those that invade the CNS and induce EAE (5). The surface marker profile of the tissue-infiltrating T cells resembles that of activated cells, and the change in these markers seen in these cited studies was attributed to the reactivation of disease-inducing T cells following their exposure to the specific Ags in the target tissue (5, 7, 8). This mechanism cannot be applied, however, to the host cells that do not recognize HEL in our system. We suggest, therefore, that the profile change of the host CD4 cells is a result of cytokines that are released by the donor cells and affect the recruited host cells. This consequent shift in surface profile is less potent than the actual activation, and, indeed, as mentioned above, the level of profile change on the host CD4 cells is markedly lower than that we observed on donor cells.

In summary, we describe in this study for the first time in detail the active involvement of host cells in the adoptively transferred Th1-mediated tissue inflammation, as well as the features of the participating cells. The transferred cells initiated spleen enlargement, as well as remarkable changes in the surface marker profile on host lymphocytes. Analysis of the recruitment process into the target tissue revealed involvement of various host cell populations, with changes of the cell surface marker profile following the migration into the target tissue. We propose that the new information on host cells in this experimental system could be applied to the population of Ag-nonspecific lymphocytes that are recruited into immune-mediated inflammatory sites.

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Disclosures

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