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CD8+ T Cells Produce the Chemokine CXCL10 in Response to CD27/CD70 Costimulation To Promote Generation of the CD8+ Effector T Cell Pool

Victor Peperzak,1 Elise A. M. Veraar,1 Yanling Xiao,2 Nikolina Bąbala,2 Klaske Thiadens,3 Marieke Brugmans,4 and Jannie Borst

Various cell types can produce the chemokine CXCL10 in response to IFN-γ stimulation. CXCL10 is generally viewed as a proinflammatory chemokine that promotes recruitment of CD8+ and Th1-type CD4+ effector T cells to infected or inflamed nonlymphoid tissues. We show that CXCL10 plays a role during CD8+ T cell priming in the mouse. Genome-wide expression profiling revealed the Cxcl10 gene as a target of CD27/CD70 costimulation in newly activated CD8+ T cells. CD27/CD70 costimulation is known to promote activated T cell survival, but CXCL10 did not affect survival or proliferation of primed CD8+ T cells in vitro. Accordingly, CXCL10 could not fully rescue CD27 deficiency in mice infected with influenza virus. Rather, CXCL10 acted as chemotactrant for other activated CD8+ T cells. It signaled downstream of CD27 in a paracrine fashion to promote generation of the CD8+ effector T cell pool in the lymph node microenvironment to facilitate their participation in the CD8+ effector T cell pool. The Journal of Immunology, 2013, 191: 3025–3036.

The T cell response is characterized by the rapid proliferation of Ag-specific T cells, their differentiation into effector cells, and their migration to the site of infection. Throughout their life, T cells rely heavily on contacts and communication with cells in their microenvironment. In lymphoid organs, fibroblastic reticular cells offer steady-state survival signals (1), whereas dendritic cells (DCs) offer the essential signals for T cell priming (2). In infected tissues, various hematopoietic and nonhematopoietic cell types produce cytokines and chemokines enabling cell–cell communication. In lymphoid organs, recently immigrated DCs produced CXCL10 to attract primed CD4+ effector T cells (9, 10). CXCR3 is also found on NK cells (11), plasmacytoid DCs (12), and activated monocytes (13). Because of the preferential expression of CXCR3 on Th1 cells, rather than Th2 cells, and the IFN-γ-inducible nature of CXCL10, this chemokine pathway is typically associated with Th1-type immune responses. Many studies report that local CXCL10 production serves to recruit effector T cells to infected or inflamed tissues. CXCL10 proved to be nonredundant with its close relatives CXCL9 and CXCL11 in promoting the accumulation of CD4+ and CD8+ effector T cells in infected tissues (14, 15). In a variety of disease models, the use of CXCL10-deficient or CXCL10-transgenic mice or the use of CXCL10-blocking Abs demonstrated that CXCL10 promotes the accumulation of CD4+ and CD8+ effector T cells in the infected, inflamed, or tumor tissues where it was produced (16–18).

However, certain studies report an effect of CXCL10 at the site of T cell priming. In CXCL10-deficient mice, the generation of CD8+ effector T cells in the spleen was impaired upon systemic virus infection (19). Likewise, generation of effector T cells in the lymph nodes upon OVA protein immunization was impaired in CXCL10-deficient mice (19). CXCL10 can influence T cell priming by enabling cell–cell communication: in lymph nodes, recently immigrated DCs produced CXCL10 to attract primed CD4+ T cells. The resulting cluster formation between the primed CD4+ T cells and the DCs promoted Th1-type effector differentiation (20). A recent report corroborated and further clarified the mechanism by...
which CXCL10 contributes to Th1 polarization during priming (21).

We report in this article that CXCL10 plays a role in CD8+ T cell priming, under control of CD27/CD70 costimulation. CD27 is a TNFR family member that is expressed on naive and activated CD4+ and CD8+ T cells (22, 23). Expression of its ligand, CD70, a TNFR family member that is expressed on naive and activated priming, under control of CD27/CD70 costimulation. CD27 is expansion of CD8+ T cells by antiapoptotic and prometabolic effects costimulation acts on other CD8+ T cells in the priming phase of survival of effector CD8+ T cells by autocrine IL-2 signaling (29). In CD4+ T cells, CD27/CD70 costimulation supports clonal expansion, as well as Th1-type effector development and help for the memory CD8+ T cell response (30, 31).

In an unbiased search for CD27-regulated transcripts in CD8+ T cells, we identified the Cxcl10 gene as one of the major CD27 target genes. In vitro studies, as well as in vivo studies with CD8+ T cells genetically reconstituted with the Cxcl10 gene, pointed out that the CXCL10 produced by CD8+ T cells in response to CD27 costimulation acts on other CD8+ T cells in the priming phase of the response. In influenza virus–infected mice, CXCL10 promoted the generation of an effector CD8+ T cell population in the lung-draining lymph node (DLNs) in a paracrine fashion. Moreover, CD8+ T cells required the CXCL10 receptor CXCR3 for their expansion in the Ag-DLNs in a peptide-immunization model that strongly relies on CD27/CD70 costimulation. Our findings suggest that the CXCL10 produced by CD8+ T cells upon CD27 costimulation recruits other CD8+ T cells to sites in the lymph node microenvironment that facilitate their participation in the CD8+ effector T cell pool.

Materials and Methods

**Mice**

Wild-type (WT), OT-I (C57BL/6-Tg[TcraTcrb]1100Mjb/J) (The Jackson Laboratory), Cd27−/− (24), OT-I: Cd27−/−, Cxcr3−/− (B6.129P2-Cxcr3tm1Jel/J), OT-I: Cd27−/−, Cxcr3−/−, F5 (32), F5; Cd27−/−, and Cd27−/−; Cd11c−/−Cd70−/− (26) mice on a C57BL/6 background were used for experiments at 7–12 wk of age. OT-I mice express a transgenic TCR with specificity for OVA257–264 peptide in the context of H-2Kb. CD8+ T cells were transferred intravenously into the tail vein of CD8a+ T cell–deficient hosts (B6.129P2-H2d−/−, F1) that were backcrossed to B6 (B6.129P2-H2d−/−, F1). Purity of the resulting OT-I T cells was 95% (Supplemental Fig. 1A). When infected, OT-I T cells were purified from nonimmunized mice were labeled with CFSE (0.5 μM), according to the manufacturer’s protocol (CellTrace; Invitrogen). Next, they were stimulated in vitro at 1 × 107 cells/well in flat-bottom plates, using 1 μg/ml coated anti-CD3 Ab, 145.2C11 (BD; cat. no. 553057), in the presence or absence of 0.5 μg/ml agonistic soluble recombinant mouse CD70-Ig fusion protein (FcCD70) (27), 0.2 μg/ml CXCX10 (PeproTech), or 20 μg/ml neutralizing rat anti-CXCL10 mAb (R&D Systems). Cell divisions and the absolute numbers of propidium iodide (PI) (live) CD8+ T cells were determined at the end of culture by cell counting and flow cytometry.

**T cell stimulation in vivo**

For gene-expression profiling, WT or Cd27−/− OT-I mice were immunized intranasally with 500 μg OVA protein and 1 μg cholea toxin (Sigma) in 50 μl HBSS (30). For the experiments depicted in Figs. 4–6, Cd27−/− F5 mice were infected intranasally with 25 hemagglutinin units of influenza virus strain A/NT/60/68, as described (24, 25, 34). For the experiments depicted in Fig. 7, Cd27−/−; Cd11c−/−Cd70−/− mice were immunized s.c. in the left flank with 20 μg OVA257–264 peptide in 100 μl PBS.

**Flow cytometry**

Cells were isolated from relevant organs, as described previously (27), and stained with fluorochrome-conjugated Abs (FITC, PE, PerCP/Cy5.5, PE/Cy7, Pacific Blue) and MHC tetramers. mAbs used were directed at CD8 (53-6.7), CD4 (L3T4 or GK1.5), CD27 (LG.3A10), anti-CD44 (IM7), anti-CXCR3, CD45.2 (53-5.2), CD4 (H12-2.1), CD11c (M1/70), CD122 (BD2), CD8 (53-6.7), and anti-CD11c (M1/70). Purity of the resulting CD8a+ T cell–deficient hosts (B6.129P2-H2d−/−, F1) that were backcrossed to B6 (B6.129P2-H2d−/−, F1). Purity of the resulting OT-I T cells was 95% (Supplemental Fig. 1A). When infected, OT-I T cells were purified from nonimmunized mice were labeled with CFSE (0.5 μM), according to the manufacturer’s protocol (CellTrace; Invitrogen). Next, they were stimulated in vitro at 1 × 107 cells/well in flat-bottom plates, using 1 μg/ml coated anti-CD3 Ab, 145.2C11 (BD; cat. no. 553057), in the presence or absence of 0.5 μg/ml agonistic soluble recombinant mouse CD70-Ig fusion protein (FcCD70) (27), 0.2 μg/ml CXCX10 (PeproTech), or 20 μg/ml neutralizing rat anti-CXCL10 mAb (R&D Systems). Cell divisions and the absolute numbers of propidium iodide (PI) (live) CD8+ T cells were determined at the end of culture by cell counting and flow cytometry.

For in vitro cultures, T cells were purified from spleens and lymph nodes, as described previously (27), and stained with fluorescein-conjugated Abs (FITC, PE, PerCP/Cy5.5, PE/Cy7, Pacific Blue) and MHC tetramers. mAbs used were directed at CD8 (53-6.7), CD4 (L3T4 or GK1.5), CD27 (LG.3A10), anti-CD44 (IM7), anti-CXCR3, CD45.2 (53-5.2), CD4 (H12-2.1), CD11c (M1/70), CD122 (BD2), CD8 (53-6.7), and anti-CD11c (M1/70). Purity of the resulting CD8a+ T cell–deficient hosts (B6.129P2-H2d−/−, F1) that were backcrossed to B6 (B6.129P2-H2d−/−, F1). Purity of the resulting OT-I T cells was 95% (Supplemental Fig. 1A). When infected, OT-I T cells were purified from nonimmunized mice were labeled with CFSE (0.5 μM), according to the manufacturer’s protocol (CellTrace; Invitrogen). Next, they were stimulated in vitro at 1 × 107 cells/well in flat-bottom plates, using 1 μg/ml coated anti-CD3 Ab, 145.2C11 (BD; cat. no. 553057), in the presence or absence of 0.5 μg/ml agonistic soluble recombinant mouse CD70-Ig fusion protein (FcCD70) (27), 0.2 μg/ml CXCX10 (PeproTech), or 20 μg/ml neutralizing rat anti-CXCL10 mAb (R&D Systems). Cell divisions and the absolute numbers of propidium iodide (PI) (live) CD8+ T cells were determined at the end of culture by cell counting and flow cytometry.

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Quantitative real-time PCR

Expression of Cxcl10 and Hprt mRNA was measured in the samples used for microarray analysis by real-time PCR (LightCycler 480 Real-Time PCR system; Roche). Fast SYBR Green Master Mix (Applied Biosystems) was used together with 10 ng cDNA template and 1 μM oligonucleotides. Levels of mRNA for the housekeeping gene Hprt were used for standardization. The oligonucleotides used to amplify the template DNA were Cxcl10 fwd: 5′-CACAACCCGTGCCTGGCATG-3′, Cxcl10 rev: 5′-GAGCTGATGTGACCAGGC-3′, Hprt fwd: 5′-CGTGTAAGGAAGCGTCTCG-3′, and Hprt rev: 5′-TGAATGCTTCAATAGCGAAGGCA-3′.

Western blotting

 Supernatants of T cells activated in vitro were harvested at the indicated time points. Protein concentration was determined using the Bio-Rad protein assay. Equal amounts of total protein/sample were separated on Novex NuPAGE 8–14% Bis-Tris Gels (Invitrogen Life Technologies), and proteins were transferred to Protran nitrocellulose membrane (Schleicher & Schuell). The membrane was blocked with 5% powdered nonfat milk (Nutricia) in TBST. Next, the membrane was incubated with biotin-conjugated goat polyclonal antiserum directed against CXCL10 (R&D Systems) in TBST with 1% nonfat milk overnight at 4°C, washed with TBST, and incubated with HRP-conjugated streptavidin for 2 h in TBST with 1% nonfat milk at 4°C. After washing, HRP-conjugated streptavidin was detected using ECL (Amer sham).

Transwell assays

T cells were purified from spleens of WT mice using a T lymphocyte enrichment set (BD) and were kept naive or were activated in vitro for 48 h with 2 μg/ml Con A (Omnimabo) in the presence of 1 ng/ml IL-7 (Peprotech). Transwells with a 5-µm pore size (Costar) were coated overnight with RetroNectin (Takara Bio, Otsu, Japan) at 50 μg/ml. Next, naive or activated T cells were added to the upper wells. IMDM or supernatants from T cells activated by aAPCs were added to the lower wells and supplemented with 0.2 μg/ml recombinant murine CXCL10 (PeproTech) or 20 μg/ml neutralizing rat anti-CXCL10 mAb (R&D Systems). T cells were allowed to migrate through the Transwell membrane for 3 h at 37°C. The migrated cells were counted with a CASY cell counter (Scharfe System) and analyzed by flow cytometry.

Retroviral transduction

The mouse Cxcl10 cDNA was obtained from the German Science Center for Genome Research and cloned into the pMXIRESGFP vector that allows bicistronic expression of the gene of interest and GFP as the result of the presence of an IRES. Empty vectors pMXIRESGFP or pMXIRESYFP were used as controls. For virus production, retroviral constructs were transfected using FuGENE 6 (Roche) into Phoenix-ECO packaging cells, together with the pCL-Eco vector encoding the ecotropic retrovirus receptor. Medium that contained retrovirus was harvested from the Phoenix-ECO packaging cells 48 h later. For retroviral transduction, splenocytes from WT and Cd27−/− F5 mice were cultured with 2 μg/ml Con A and 1 ng/ml rIL-7 for 48 h. Next, they were resuspended at 2 × 10^6 cells/ml retrovirus-containing medium and placed in nontissue culture–treated 24-well plates (BD) coated with 50 μg/ml RetroNectin (Takara Bio). Plates were spun for 90 min at 450 × g, and cells were cultured for 20 h prior to their use for adoptive transfer.

Adoptive transfer

In the influenza virus–infection experiments, Cxcl10-transduced or empty vector–transduced splenocytes of WT and Cd27−/− F5 mice were incubated with allophtocyanin-conjugated anti-CD8 mAb in IMDM with FCS for 30 min on ice. Cells were washed and resuspended in IMDM with FCS and sorted by flow cytometry for GFP or YFP and CD8. The resulting purified transduced CD8+ T cells were injected retro-orbitally at 5 × 10^5 cells/mouse in 100 μl HBSS. In the OVA protein or OVA peptide–immunization experiments, OT-I, OT-2/Cxcl10−/−, and OT-I/Cxcr3−/− GFP cells were purified with the iMag mouse CDS T Cell Enrichment Set (BD). OT-I T cells were injected retro-orbitally at 1 × 10^5 cells/genotype in 100 μl HBSS/mouse.

Statistical analysis

Statistical significance was determined using a two-tailed Student t test.

Results

Primed CD8+ T cells produce CXCL10 in response to CD27/CD70 costimulation

We performed genome-wide mRNA expression profiling to identify the molecular mechanisms by which CD27/CD70 costimulation supports the CD8+ T cell response. For this purpose, in vitro and in vivo experimental settings were used in which CD27/CD70 costimulation was the only variable. We used OT-I TCR transgenic T cells that recognize OVA257–264 peptide in the context of H-2Kb as responder CD8+ T cells. In vitro, purified OT-I T cells (Supplemental Fig. 1A) were activated by coculture with aAPCs in the form of mouse embryo fibroblasts that present OVA257–264 peptide and did or did not express CD80 (33). In the two comparative settings, we stimulated WT OT-I cells with aAPCs that did or did not express CD70 or we stimulated WT and Cd27−/− OT-I T responder cells with aAPCs that expressed CD70 (Fig. 1A). This way, we selectively monitored the effect of CD27/CD70 costimulation in the presence or absence of CD28/CD80 costimulation. After 2, 4, 8, or 14 h of stimulation, the OT-I T cells were removed from the adherent aAPCs, purified further (Supplemental Fig. 1A), and used for RNA extraction. In vivo, WT or Cd27−/− OT-I T cells were activated by immunizing the mice intranasally with OVA protein. After 3, 4, or 8 d, the OT-I T cells were purified from lung-DLNs, spleen, or lung by flow cytometric sorting, based on H-2Kb/OVA257–264 tetramer and CD8 staining (Supplemental Fig. 1B). The mRNA isolated from the OT-I T cells at these time points was analyzed with microarrays that represent 72% of all known mouse genes (29, 30). Genes regulated by CD27/CD70 costimulation were selected on the stringent criteria of differential expression with a p value < 0.00003 in at least two experimental settings. A heat map was constructed that included in the hierarchy the fold differential expression and the number of experimental settings in which the gene was differentially expressed (Supplemental Fig. 2).

In the comparative setting of WT versus Cd27−/− OT-I cells, the Cxcl10 gene was consistently identified as the most important differentially expressed gene, confirming the validity of the approach (Supplemental Fig. 2). The Il-2 gene was at the top of the hierarchy of CD27-regulated genes, as previously reported (29). At the indicated p value, this analysis revealed ∼30 genes that were differentially expressed (Supplemental Fig. 2). To our surprise, the Cxcl10 gene was number five on this hit list of potential CD27 target genes in primed CD8+ T cells. CD27/CD70 costimulation induced Cxcl10 mRNA expression both in vitro and in vivo (Fig. 1A). Quantitative real-time PCR confirmed the rapid upregulation of Cxcl10 mRNA expression within 4 h after CD27 costimulation in both in vitro settings (Fig. 1B). CD27 costimulation also upregulated Cxcl10 mRNA levels in a transient manner when polyclonal CD8+ T cells were activated with anti-CD3 mAb (Supplemental Fig. 3A). This upregulation still occurred when protein synthesis was blocked, indicating that CD27 signaling directly promoted Cxcl10 gene transcription and/or mRNA stability (Supplemental Fig. 3B).

To verify that CXCL10 was differentially expressed at the protein level, medium was harvested from cultures of WT and Cd27−/− OT-I cells that had been stimulated for 48, 72, or 96 h with CD70+ aAPCs, and Western blotting was performed (Fig. 1C). The levels of CXCL10 protein were higher in the medium of WT OT-I T cells than that of Cd27−/− OT-I T cells at all time points tested, apparently due to the transient increase in Cxcl10 mRNA levels as result of CD27 costimulation. CD27/CD70 costimulation is known to promote clonal expansion of T cells, but the numbers of live WT and Cd27−/− OT-I T cells were not significantly different at the 48- and 72-h time points (Fig. 1D), in-
indicating that WT OT-I T cells produced more CXCL10 on a per-cell basis. We conclude that CD27 costimulation of primed CD8+ T cells increases CXCL10 protein expression by effects at the mRNA level. The protein synthesis–independent upregulation of Cxcl10 mRNA by CD27 strongly suggests that CD27 signaling directly stimulates Cxcl10 gene expression and/or mRNA stability.

CXCL10 produced upon CD27/CD70 costimulation attracts activated CD8+ T cells

To examine which T cell subsets in priming organs might be responsive to CXCL10, we determined the expression of CXCR3, the unique receptor for CXCL10. In agreement with published results (35), a fraction of CD4+ and CD8+ T cells in spleen and inguinal lymph nodes of nonimmunized mice expressed CXCR3 (Fig. 2A, 2B). Most CXCR3+ CD8+ T cells had a central memory phenotype, whereas CXCR3+ CD4+ T cells predominantly had an effector (memory) phenotype (Fig. 2C, 2D). In addition, a proportion of naive phenotype CD4+ and CD8+ T cells expressed CXCR3 (Fig. 2C, 2D). In concordance with the predominant presence of CXCR3 on Ag-experienced cells, most splenic CD8+ T cells acquired CXCR3 expression upon activation with Con A in vitro (Fig. 2E), before acquisition of a CD62Llow effector phenotype (data not shown). However, splenic CD4+ T cells did not upregulate CXCR3 expression upon activation with Con A in vitro (Fig. 2E).

In vitro Transwell assays were performed to assess T cell migration in response to CXCL10 or potentially other factors produced in response to CD27 costimulation. Polyclonal CD8+ T cells isolated from the spleens of nonimmunized mice migrated toward recombinant murine CXCL10 after Con A activation, and this was completely blocked by a neutralizing anti-CXCL10 mAb, thus validating the assay (Supplemental Fig. 3C). Splenic CD4+ T cells did not detectably respond to CXCL10, either before or after Con A activation (Supplemental Fig. 3C), in agreement with lack of CXCR3 upregulation (Fig. 2E). Next, we tested the effect of soluble factors, produced by OT-I T cells in response to CD27/CD70 costimulation, on the migration of activated polyclonal CD8+ T cells. For this purpose, we used culture medium from WT or Cd27−/− OT-I T cells that had been activated for 48 h by CD70-expressing aAPCs. Medium from WT OT-I T cells attracted more activated CD8+ T cells than did medium from Cd27−/− OT-I T cells (Fig. 2F), in agreement with the larger amount of CXCL10 present (Fig. 1C). Addition of CXCL10-neutralizing mAb to the medium of WT OT-I T cells strongly inhibited the migration of polyclonal CD8+ T cells (Fig. 2F). By mAb-mediated blocking of CXCL10, the migratory response toward WT OT-I T cell medium became comparable to the migratory response toward Cd27−/− OT-I T cell medium (Fig. 2D). Therefore, we conclude that CXCL10 is the only chemoattractant for activated CD8+ T cells that is produced by CD8+ T cells in response to CD27/CD70 costimulation.

CXCL10 does not affect survival or clonal expansion of primed CD8+ T cells in vitro

CD27/CD70 costimulation is known to promote the survival of primed CD8+ T cells. In lymph nodes, CD27/CD70 costimulation induces expression of Bcl-xL and the Pim-1 kinase that provide antiapoptotic and prometabolic signals to CD8+ T cells during clonal expansion (27). It was suggested that CXCL10 can promote T cell proliferation (36). For this reason, we tested the potential
impact of CXCL10 on clonal expansion and survival of primed CD8+ T cells in vitro. WT and Cd27−/− polyclonal CD8+ T cells were activated by low-level TCR/CD3 triggering with anti-CD3 mAb and costimulated via CD27 with a soluble recombinant CD70 protein (FcCD70) (27, 29). Cell division was read out by dilution of CFSE, and cell survival was assessed by uptake of PI. In these cultures, CD27 costimulation with FcCD70 greatly increased the yield of live WT CD8+ T cells at day 3 of culture (Fig. 3A). FeCD70 did not have any effect on Cd27−/− CD8+ T cells, demonstrating that it acted by engaging CD27 (Fig. 3A). Recombinant CXCL10 or neutralizing anti-CXCL10 mAb did not influence the yield of live WT or Cd27−/− CD8+ T cells, either in the presence or in the absence of FeCD70 at day 3 of the response (Fig. 3A) or at day 4 (data not shown).
CXCL10 promotes CD8+ effector T cell generation

Under conditions of low TCR/CD3 input, CD27/CD70 costimulation is known to promote cell cycle entry of murine CD8+ T cells, most likely as the result of survival signaling (27). This was also revealed in the current assay by CFSE dilution. With TCR/CD3 stimulation alone, only a very small proportion of cells had divided at day 3, whereas in the presence of CD27 costimulation, the great majority of cells had entered division and completed multiple cycles (Fig. 3B, no treatment versus FcCD70). Addition of CXCL10 had no effect on CD8+ T cell cycling, nor did the addition of neutralizing anti-CXCL10 mAb, either in the absence or in the presence of CD27 costimulation (Fig. 3B). Therefore, we conclude that, in vitro, survival and clonal expansion of CD8+ T cells, driven by TCR/CD3 and CD27 signaling, does not rely on CXCL10.

Reconstitution of the Cxcl10 gene in Cd27−/− CD8+ T cells improves in vivo priming

Next, we determined how CXCL10 contributed to CD27 function in vivo. For this purpose, we used influenza virus infection, a model that we used previously to delineate the contribution of CD27/CD70 costimulation to the CD8+ T cell response (24, 25, 34). As responders, we used CD8+ T cells of the F5 TCR transgenic mouse strain that recognize the immunodominant NP68-75 epitope in the context of H-2Dk. Our aim was to determine to what extent reconstitution of Cxcl10 gene expression could rescue the defects that Cd27−/− CD8+ T cells display in this model system. WT and Cd27−/− F5 T cells were transduced to express the Cxcl10 gene or empty control vector in an IRES-GFP configuration that enabled flow cytometric purification of transduced F5 T cells. These cells were adoptively transferred into Cd27−/− recipient mice that were subsequently infected with influenza virus (Fig. 4A).

At days 7 and 8 postinfection, mediastinal-DLNs and spleen of recipient mice were analyzed for the number of F5 T cells, using GFP and CD8 as identifiers. At both days 7 and 8, Cd27−/− F5 T cells with empty vector showed a reduced accumulation compared with WT F5 donor cells with empty vector in DLNs (Fig. 4B), as well as to some extent in the spleen (Fig. 4C). This was expected because CD27/CD70 costimulation promotes clonal expansion and survival of primed CD8+ T cells, particularly in the DLNs in this model system (25, 27). In the case of WT F5 T cells, Cxcl10 gene transduction did not alter the response in DLNs or spleen (Fig. 4B, 4C). However, in case of Cd27−/− F5 T cells, Cxcl10 gene transduction significantly improved accumulation in the DLNs at day 8 postinfection (Fig. 4B), but the response was still deficient compared with WT cells. In the spleen, the response of Cd27−/− F5 T cells was not significantly affected by Cxcl10 gene transduction (Fig. 4C). Western blotting validated the expression of CXCL10 protein from the retroviral construct (Fig. 4D).

In conclusion, Cxcl10 gene transduction did not affect the response of WT CD8+ cells, indicating that it specifically complemented defects of Cd27−/− CD8+ T cells. As expected from the fact that CD27 acts also via Bcl-xL and Pim-1 during CD8+ T cell priming (27), Cxcl10 gene transduction could not fully rescue the defects in clonal expansion and accumulation of Cd27−/− responder CD8+ T cells. However, it did improve their clonal expansion in the DLNs.

CXCL10 acts in a paracrine fashion to promote the generation of CD8+ effector T cells

In the same experiment, we also determined how Cxcl10 gene transduction in donor F5 T cells affected the T cell response of the Cd27−/− recipient mice. This was done by enumerating CD8+ and CD4+ T cells that had acquired a CD62Llow effector phenotype. At day 8 postinfection, generation of CD8+ CD62Llow effector T cells in the DLN was significantly lower in mice that had received Cd27−/− F5 donor T cells with empty vector compared with mice...
that had received WT F5 donor T cells with empty vector (Fig. 5A). A similar phenotype was observed for CD4+ effector T cell generation (Fig. 5B). This result indicated that CD27 deficiency of the F5 donor T cells impacted on the CD8+ and CD4+ T cell responses of the (Cd27−/−) recipient mice. When Cd27−/− F5 donor T cells had been transduced to express the Cxcl10 gene, they improved the CD8+ T cell response of the recipients to the level observed in mice that had received WT F5 donor T cells transduced with empty vector (ev) encoding GFP only. Two days later, transduced F5 T cells were sorted for CD8 and GFP expression and adoptively transferred (5000 cells/mouse) into Cd27−/− recipients, which were infected with influenza virus on the following day. At day 7 or 8 post-infection, cells were harvested from DLNs and spleen, enumerated, and analyzed by flow cytometry. Absolute numbers of GFP+ CD8+ F5 T cells of the indicated genotypes recovered from DLNs (B) or spleen (C) at days 7 and 8. Data are mean ± SEM (n = 4 mice/group). The experiment is representative of three independent experiments. (D) Verification of CXCL10 protein expression by transduced Cd27−/− F5 cells before adoptive transfer. Western blotting with anti-CXCL10 mAb was performed on supernatant of F5 T cells transduced with empty vector (ev) or Cxcl10-IRES-GFP vector. *p < 0.05, Student t test.
To examine the paracrine effect of CXCL10 on the CD8+ T cell response of the recipient mice, the absolute numbers of recipient CD62Llow effector CD8+ T cells were determined at days 6 and 8 postinfection. At day 8, the number of recipient CD8+ effector T cells formed in DLNs (A) or spleen (B) of Cd27−/− mice that had received donor F5 T cells of the indicated genotypes. (B and C) CD4+ T cell responses. Absolute numbers of recipient CD62L effector CD8+ T cells formed in DLNs (C) or spleen (D) of Cd27−/− mice that had received donor F5 T cells of the indicated genotypes. Data are mean ± SEM of four mice/group. The experiment is representative of three independent experiments.

CXCL10 gene transduction cannot rescue CD27-deficient T cell responses at the effector site

In this model of influenza virus infection, the lung is a bona fide tissue effector site. There is no formation of BALT and no priming of T cells in the lung (34) (data not shown). All CD4+ and CD8+ T cells that accumulate in the lung have an effector phenotype, and the accumulation occurs after T cell priming in the mediastinal DLNs (24, 25, 34). We showed previously that CD27 costimulation provides an autocrine IL-2–signaling pathway that is highly dependent on CD27/CD70 costimulation (26). Therefore, this model optimally reveals the contribution of CD27/CD70 costimulation during the priming of CD8+ T cells. CD11c-Cd70 transgenic mice that constitutively express CD70 on DCs. When immunized with MHC class I–restricted peptide in PBS, a situation that is ordinarily tolerogenic, these mice mount a CD8+ T cell response that is highly dependent on CD27/CD70 costimulation during the priming of CD8+ T cells. CD11c-Cd70 transgenic mice are maintained on a Cd27−/− background to avoid the effects of transgenic CD70 on the endogenous T cells.

CD8+ T cells require CXCR3 expression to optimally profit from CD27/CD70 costimulation

Because CXCL10 exerts its functional effects via CXCR3, it followed from our observations that the effects of CD27/CD70 costimulation on CD8+ T cells must rely, in part, on CXCR3 expression. To test this, we made use of CD11c-Cd70 transgenic mice that constitutively express CD70 on DCs. When immunized with MHC class I–restricted peptide in PBS, a situation that is ordinarily tolerogenic, these mice mount a CD8+ T cell response that is highly dependent on CD27/CD70 costimulation during the priming of CD8+ T cells. CD11c-Cd70 transgenic mice are maintained on a Cd27−/− background to avoid the effects of transgenic CD70 on the endogenous T cells.

We previously used i.v. immunization with peptide; however, in this case, we aimed to examine effects in the DLNs. Therefore, we tested the model with s.c. peptide immunization. WT and Cd27−/− OT-I donor T cells were transferred at equal ratios into the same...
Cd27−/−:Cd70tg recipient mice that were subsequently immunized s.c. with OVA257–264 peptide in PBS in the left flank. The OT-I T cell response was read out over the next 13 d in blood (Fig. 7A). Only the CD27-proficient OT-I T cells were able to respond, thereby validating that the system is reliant on CD27/CD70 co-stimulation (Fig. 7B).

Next, WT and Cxcr3−/− OT-I T cell responses were compared in this setting by transfer of both cell types into the Cd27−/−:Cd70tg recipient mice. In the blood, the Cxcr3−/− OT-I T cell response was significantly lower than the WT OT-I T cell response, but it was higher than the response of Cd27−/− OT-I T cells (Fig. 7B). Moreover, in the left inguinal–DLN on day 7, the Cxcr3−/− OT-I T cell response was significantly lower than the WT OT-I T cell response (Fig. 7C). There was also a significant difference in the right inguinal lymph node, whereas the responses in the spleen were not significantly different. From these results, we conclude that, in a CD27/CD70-dependent response, the accumulation of Ag-specific CD8+ T cells in the DLNs is partially dependent on their expression of CXCR3. Furthermore, these data support the idea that CXCL10, produced by CD8+ T cells in response to CD27/CD70 costimulation, promotes effector CD8+ T cell accumulation at the site of priming by acting directly on Ag-specific CD8+ T cells.

Discussion

In this study, we found that CD27/CD70 costimulation induces Cxcl10 gene transcription in mouse CD8+ T cells. In response to CD27 triggering, CD8+ T cells upregulated Cxcl10 mRNA expression with rapid kinetics, both in the presence and absence of CD28 costimulation. Moreover, CD27 costimulation increased Cxcl10 mRNA levels independent of new protein synthesis. This strongly suggests that Cxcl10 is a CD27 target gene. CD27/CD70 costimulation also induced Cxcl10 gene transcription and protein production in human CD8+ T cells (data not shown). The conserved rapid upregulation of CXCL10 expression suggested an important contribution of this chemokine during the priming phase of the CD8+ T cell response.

Cxcl10 gene transduction improved, but did not fully correct, the deficient clonal expansion of Cd27−/− F5 T cells in DLNs...
upon in vivo challenge with influenza virus, in agreement with the
known mechanism of action of CD27. CD27 can promote clonal
expansion of CD8+ T cells at the site of priming by survival sig-
naling (25). This proceeds, at least in part, via Bcl-xL and Pim-1,
which exert cell-autonomous antiapoptotic and prometabolic
effects (27). Accordingly, CD27 costimulation promoted the TCR/
CD3-driven clonal expansion of primed CD8+ T cells in vitro,
independent of CXCL10. CD27 costimulation also supports the
survival of effector CD8+ T cells after the clonal expansion phase
via autocrine IL-2 signaling (29). The Il2 gene is the most im-
portant target gene of CD27 costimulation in primed CD8+ T cells
(29) (Supplemental Fig. 2). The IL-2 produced in response to
CD27 costimulation does not contribute to clonal expansion of
primed CD8+ T cells in lymphoid organs, but it supports the
survival of CD8+ effector T cells in nonlymphoid tissue (29).
In fact, the defective accumulation of Cd27−/− CD8+ effector T
cells in the lung of influenza-infected mice could be fully rescued by
Il2 gene transduction. This indicates that survival signaling via IL-2 is
the main mechanism by which CD27 supports accumulation of
CD8+ effector T cells at the tissue site. In agreement, Cxcl10 gene
transduction could not rescue the survival defect of Cd27-deficient
CD4+ and CD8+ T cells in the lung after influenza virus infection.

Our current findings highlight that CD27 costimulation promotes
the CD8+ T cell response by effects on T cell survival, as well as
by the induction of chemokine expression, which we propose
serves to optimize the cellular niche for T cell priming.

Members of the TNFR family are generally implicated in sur-
vival signaling. Via TRAF adaptor molecules, CD27 and other
TNFR family members link to the NF-kB–signaling pathway (37–
40), which mediates expression of various antiapoptotic molecules
(41). However, the NF-kB pathway can also induce the expres-
sion of chemokines, including CXCL10 (42, 43). From this perspec-
tive, it is perhaps not surprising that CD27 directs
Cxcl10 gene expression. In contrast, the only TNF family members that
have been implicated in CXCL10 expression are TNF-
a (44), CD40L
(45, 46), and TWEAK (47), all in nonlymphoid cell types. The
finding that a T cell costimulatory receptor induces chemokine
expression during priming is novel and begs for a conceptual in-
terpretation. Simultaneous induction of prosurvival molecules and
chemokines by TNFR family members suggests that these re-
ceptors create specific cellular niches that may support cell pro-
liferation and/or differentiation. Our data indeed suggest that the
CXCL10 produced in response to CD27 costimulation acts in
dedicated cellular niches. CXCL10 production by CD8+ responder

FIGURE 7. CD8+ T cells require CXCR3 expression for clonal expan-
sion in a CD27/CX70-dependent model of peptide immunization. (A)
Experimental design. WT OT-I (CD45.1) and Cd27−/− OT-I (CD45.2) or WT OT-I
(CD45.1) and Cxcr3−/− (GFP+) OT-I T cells were adoptively transferred, at
a 1:1 ratio of 10,000 cells/genotype, into Cd27−/−;Cdh70g recipient mice
that transgenically express CD70 on steady-state DCs. Mice were challenged
s.c. in the left flank with OVA257-264 peptide in PBS 1 d later. OT-I T cell
responses were read out subsequently in blood on days 0–13 and the left and
right inguinal lymph node on day 7. (B) Comparison of WT and Cd27−/− OT-I
T cell responses. At the indicated days after immunization, the percentages of
CD8+ H-2k2/OVA257-264 tetramer+ OT-I T cells of WT (CD45.1+) or Cd27−/−
(CD45.2+) origin were determined in the blood. (C and D) Comparison of
WT and Cxcr3−/− OT-I T cell responses. (C) Percentages of WT (CD45.1+) or
Cxcr3−/− (GFP+) CD8+ OT-I T cells were determined in the blood at the
indicated days after immunization (right panel). Data are mean + SEM of three
mice/group, and the experiment is represent-
tative of two experiments. (D) On
day 7, the percentages of WT (CD45.1+)
or Cxcr3−/− (GFP+) CD8+ OT-I T cells
were determined in the left and right
inguinal lymph nodes (LN) and in the
spleen. Data are mean + SEM of five
mice/group, and the experiment is rep-
tative of two experiments. *p <
0.05, Student ttest.
T cells improved the virus-specific CD8+ T cell response in the DLNs but not in the spleen. We hypothesize that CXCL10 produced by responder CD8+ T cells can only impact on formation of the CTL effector pool by local effects in the lymph node micro-environment.

Chemokines play an important role in the lymph nodes. Under conditions of homeostasis, certain chemokines orchestrate the separation of T and B cells in different zones. Upon infection, de novo expressed chemokines and their receptors direct the migration of T and B cells to optimize specific cellular interactions (48). For instance, naive CCR7+ T cells remain in T cell zone under homeostatic conditions, but CCR4 receptor expression allows activated T cells to migrate toward newly arrived DCs in the paracortex (49). Along the same lines, Yoneyama et al. (20) found that CXCL10 recruits primed CD4+ T cells from the T cell zone to recently immigrated DCs in the paracortex of hepatic lymph nodes after i.v. immunization with heat-killed bacteria. They postulated that sustained contact of the already primed, but uncommitted, CD4+ T cells with the CXCL10-producing DCs in the paracortex of the lymph node allowed for expansion and full Th1 polarization. Indeed, a recent study proves this point (21). CXCR3 was upregulated on CD4+ T cells in DLNs during priming, prior to clonal expansion, and contributed to migration of Ag-specific CD4+ T cells from the T cell zone to the interfollicular and medullary regions, to interaction with DCs, and to Th1 differentiation. CXCL10 was expressed in the medulla of the DLNs and in scattered cells in the T cell zone, and CXCL10 produced by DCs and possibly other hematopoietic cells was required for optimal Th1 cell formation (21). These studies were the first to implicate cell migration directed by CXCL10 in the fate of Ag-specific T cells at the priming site, in this case CD4+ T cells. We now add that CXCL10 performs such a function for CD8+ T cells, in accordance with a recent finding that CXCL10 produced by DCs can support the generation of a CD8+ T cell response (50).

Interestingly, recent findings on the role of CXCR3 in fate determination of primed CD8+ T cells further support this notion (51, 52). CXCR3 binds CXCL9, CXCL10, and CXCL11, but C57BL/6 mice only express CXCL9 and CXCL10 (52). Therefore, findings on CXCR3 function in C57BL/6 mice reflect the role of one or both of these chemokines. Kurachi et al. (51) and Hu et al. (52) found that CXCR3 on CD8+ T cells promoted their commitment to an effector fate, rather than a memory fate. Both studies used acute infection with a systemic virus and monitored CD8+ T cell responses in the spleen. CXCR3 was rapidly upregulated on CD8+ T cells during the first few days of priming, and its expression affected the localization of CD8+ T cells within the spleen in the priming phase. WT CD8+ T cells were initially found in the T zone, but they relocalized to the marginal zone where they formed clusters with DCs. This redistribution was impaired when the CD8+ T cells lacked expression of CXCR3. It was suggested that this redistribution within the priming organ and the consequent longer or shorter contact with Ag-presenting DCs determined the long-term fate of the CD8+ T cells (51, 52).

Our data are in full agreement with the concept that CXCR3 and CXCL10 play a role in the localization of primed CD8+ T cells at the site of priming and, thereby, affect the CD8+ T cell response. CXCL10 produced by primed CD8+ T cells in response to CD27/CD70 costimulation may serve to attract recently primed CD8+, either to profit from each other’s company or to profit from sustained colocalization with Ag-presenting DC, which support clonal expansion and effector cell formation. Our data indicate that primed CD4+ T cells can also profit from CXCL10 production by primed CD8+ T cells. Notably, gene-expression profiling also revealed that CD27 induced expression of CCL4 and XCL1 in primed CD8+ T cells (Supplemental Fig. 2), which suggests attraction of CD4+ T cells (53) and cross-priming DCs (54). This is in perfect agreement with known functions of CD27 in priming at the T cell–DC interface (26, 55) and orchestrating CD4+ T cell help for the CD8+ T cell response (29, 55, 56). We did not find a significant phenotype for CXCL10 or CXCR3 expression in the spleen, which is in line with the localized nature of Ag delivery, wherein the DLN is the first site that receives the Ag from the site of infection or immunization.

In conclusion, our data substantiate that CXCL10 plays a role in the priming phase of the T cell response. We present the novel concept that a T cell costimulatory receptor induces the synthesis of a chemokine to influence T cell fate. CXCL10 production by primed CD8+ T cells does not directly affect CD8+ T cell survival or proliferation, yet it impacts on effector T cell generation in vivo. These findings connect very well with the recently identified role for CXCR3 in effector development of CD8+ T cells and emphasize that chemokines can indirectly affect cell fate by orchestrating cell–cell communication.

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Disclosures

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