GAATTCTCAGCTTCCTGCTAATCA-3′) and $J_{\kappa 5}$ primer (5′-ATAGGATCCCGTTTCAGCTCCAGCTTGGT-3′) for 20 cycles, followed by an additional 30 cycles with (5′-GCCGGAATTCCCAGAGGACAAATTGTTC-3′)]. The cDNA PCR products were purified with Magic PCR Preps (Promega) and sequenced with a $J_{\kappa 5}$-specific primer (5′-ACGTTTCAGCTCCAGCTTGGTC-CCA-3′). DNA sequencing was performed with T7 DNA polymerase or with Taq polymerase with the use of di- or dye-deoxy terminators.

16. The source of CD40 ligand was the transfectant cell line K47 (G. Wohlleben *et al.*, *Int. Immunol.*, in press) that constitutively expresses CD40 ligand (CD40L).
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18. T cell–depleted splenic B cells from BALB/c mice immunized with phOx-OVA, as described above (*15*), were stimulated with the indicated stimuli in 96-well plates at the indicated cellular input. After 8 days in culture, the supernatants were screened for phOx reactivity by ELISA, the positive wells were scored, and the cells were harvested. The $V_{\kappa Ox1}$ rearrangements were amplified by RT-PCR as described above and cloned into pGem3Z vector. Random plasmids containing inserts were selected and sequenced. The measured Taq polymerase error under these conditions is less than one mutation per 1000 bases sequenced.
19. Supported by grants from the Swedish Medical Research Council, the Alfred Österlund Foundation, the Crafoord Foundation, the Swedish Cancer Society, the British Medical Research Council, Kocks Foundation, and the Wellcome Trust.

9 October 1995; accepted 8 December 1995

Role of Lymphotoxin and the Type I TNF Receptor in the Formation of Germinal Centers

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In mice deficient in either lymphotoxin-α (LT-α) or the type I tumor necrosis factor (TNF) receptor, but not the type II TNF receptor, germinal centers failed to develop in peripheral lymphoid organs. Germinal center formation was restored in LT-α–deficient mice by transplantation of normal bone marrow, indicating that the LT-α–expressing cells required to establish this lymphoid structure are derived from bone marrow.

The structurally related cytokines LT and TNF-α can modulate many immune and inflammatory reactions (*1*). LT has been identified in two different molecular forms: a secreted form consisting of an LT-α homotrimer and a membrane-associated heteromeric complex, composed (in its major form) of one LT-α monomer and two identical 33-kD transmembrane LT-β subunits (2–4). The secreted LT-α homotrimer binds and activates both TNF receptor type 1 (TNFR-I) and type 2 (TNFR-II) (*5*), whereas the membrane-associated $LT\text{-}\alpha_1$–$LT\text{-}\beta_2$ heteromer engages a newly identified receptor designated the TNF receptor–related protein (TNFRrp) (*4*, *6*). In vitro, LT duplicates the activities of TNF-α. However, recent studies have demonstrated that LT-α occupies a central, unique role in the development of lymph nodes (LNs) and Peyer's patches (PPs) (*7*). The relative roles of TNFR-I, TNFR-II, and other LT-binding cell surface receptors in transducing LT-specific signals have not been defined (*5*).

Mice rendered deficient in LT-α ($LT\text{-}\alpha^{-/-}$) are born without morphologically detectable LNs or PPs (*7*). The spleen, although present, shows disturbed architecture, with failure in the white pulp of normal segregation of B and T cell zones and failure to establish a distinct marginal zone. These findings suggest that responses that depend on normal cellular interactions within white pulp follicles may be dysfunctional in these mice.

We tested this hypothesis by immunizing normal and $LT\text{-}\alpha^{-/-}$ mice with sheep red blood cells (SRBCs) (*8*). Ten days after immunization, immunohistochemical evaluation of the spleens of wild-type mice demonstrated the formation of prominent germinal centers (GCs), visualized as clusters of cells binding peanut agglutinin (PNA) (*9*) surrounded by cells staining with antibody to immunoglobulin D (IgD) (Fig. 1A). In contrast, spleen sections from $LT\text{-}\alpha^{-/-}$ mice immunized with SRBCs showed no GC structures (Fig. 1B). PNA-binding cells were rarely detected and, when seen, were closely approximated to vascular structures and not associated with IgD-expressing cells. Similar results were obtained after immunization with (4-hydroxy-3-nitrophenyl)acetyl–ovalbumin and trinitrophenyl keyhole limpet hemocyanin. These observations indicate that in addition to supporting the development of LNs and PPs, LT-α has an essential function in supporting the generation of normal GC structure.

After their release in soluble form from cells, both TNF-α and LT-α are homotrimers (*10*). These two cytokines can engage both the 55-kD TNF receptor (TNFR-I) and the 75-kD TNF receptor (TNFR-II) (*3*, *5*). To evaluate whether either of these TNF receptors was required for normal GC formation, we used SRBCs to immunize mice rendered deficient in either TNFR-I ($TNFR\text{-}I^{-/-}$) or TNFR-II ($TNFR\text{-}II^{-/-}$) by gene targeting (*11*). Although $TNFR\text{-}II^{-/-}$ mice had morphologically normal GCs (Fig. 1D), $TNFR\text{-}I^{-/-}$ mice developed no GCs (Fig. 1C), demonstrating that signaling through TNFR-I is also required for GC development. Experiments performed in vitro (*12*) and in vivo (*13*) have suggested unique activities for TNFR-I and TNFR-II. Previous studies of mice rendered deficient in each of these receptors by gene targeting have also iden-

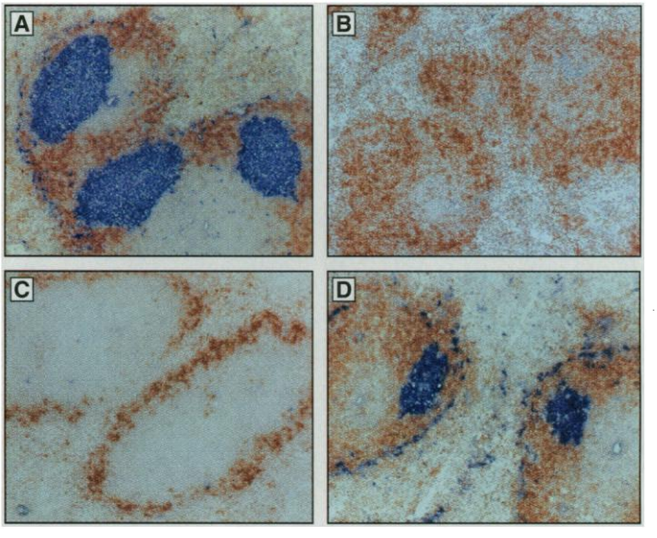

Fig. 1. Absence of GCs in spleens from immunized $LT\text{-}\alpha^{-/-}$ and $TNFR\text{-}I^{-/-}$ mice but not from $TNFR\text{-}II^{-/-}$ mice. Spleen sections from wild-type (**A**), $LT\text{-}\alpha^{-/-}$ (**B**), $TNFR\text{-}I^{-/-}$ (**C**), and $TNFR\text{-}II^{-/-}$ (**D**) mice were stained with PNA (blue) and antibody to IgD (brown). Typical GC development is absent in $LT\text{-}\alpha^{-/-}$ and $TNFR\text{-}I^{-/-}$ mice, but present in wild-type and $TNFR\text{-}II^{-/-}$ mice after immunization with the T lymphocyte–dependent antigen SRBC. Magnification, ×30.

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tified phenotypic differences, suggesting a dominant role for TNFR-I in resistance to *Listeria monocytogenes* and in the lethal response to endotoxin plus D-galactosamine (*14*). In this regard, the requirement for TNFR-I but not for TNFR-II in the formation of GCs provides additional clear evidence that signals from these two TNFRs are not redundant in vivo.

To evaluate more fully the alterations of spleen follicular structure in LT-$\alpha^{-/-}$ and TNFR-I$^{-/-}$ mice, we stained tissue sections from the spleens of immunized mice with MOMA-1 (*15*), a monoclonal antibody specific for the metallophilic macrophages that constitute a major component of the marginal zone (*16*). The pattern of MOMA-1 staining in TNFR-I$^{-/-}$ mice was indistinguishable from that in wild-type and TNFR-II$^{-/-}$ mice (Fig. 2, A, C, and D). In contrast, MOMA-1 staining was essentially absent in the spleens of LT-$\alpha^{-/-}$ mice (Fig. 2B). This indicates that although both LT-α and TNFR-I play essential roles in the generation of GCs, they do not mediate identical actions in the development of mature lymphoid follicles.

In previous studies, we investigated the mechanism of failure of LN formation in LT-$\alpha^{-/-}$ mice through use of reciprocal bone marrow transfers (*17*). These studies demonstrated that LT-$\alpha^{-/-}$ lymphoid cells could repopulate the LNs of irradiated normal mice as effectively as could wild-type lymphoid cells. In contrast, wild-type lymphoid cells, although able to repopulate the spleen, could not induce the de novo development of LN tissue in irradiated LT-$\alpha^{-/-}$ adult mice. This suggested that LT-α was not required for the targeting of bone marrow–derived cells to existing LNs, but rather that LT-α controlled a cellular interaction required for LN histogenesis and that the LN defect was developmentally fixed. To determine whether the defect in GC formation was similarly developmentally fixed, we immunized the bone marrow–chimeric mice with SRBCs and analyzed spleen sections by staining with PNA and antibody to IgD (*18*). Reconstitution of irradiated LT-$\alpha^{-/-}$ mice with normal bone marrow restored the ability to form GCs (Fig. 3A). In contrast, when LT-$\alpha^{-/-}$ bone marrow was used to reconstitute irradiated wild-type mice, GC formation was defective as in the LT-$\alpha^{-/-}$ mice (Fig. 3B). Thus, the abnormal spleen architecture observed in LT-$\alpha^{-/-}$ mice is a plastic characteristic determined by the bone marrow–derived cells that populate the spleen, rather than a fixed characteristic defined by the spleen stroma. These data further indicate that bone marrow–derived cells are the only essential source of LT-α required for the formation of GCs.

Although targeting either the gene encoding LT-α or TNFR-I blocked formation of GCs, there were differences in lymphoid follicle morphology in these two targeted strains. The distribution of MOMA-1 staining was apparently normal in TNFR-I$^{-/-}$ and TNFR-II$^{-/-}$ mice. In contrast, MOMA-1 staining was essentially absent in LT-$\alpha^{-/-}$ mice. This establishes a role for LT-α in the formation of a morphologically normal marginal zone, apparently acting through a receptor other than TNFR-I or TNFR-II. We speculate that, as suggested for development of LNs and PPs, the development of normal marginal zone structure is dependent on membrane LT, acting through TNFRrp (6) or a similar receptor.

Complete GC structure is expressed after exposure to antigen, which activates LT-α expression in T helper 1 lymphocytes (*19*). Thus, LT-α could stimulate GCs directly after T helper cell activation. Alternatively, LT-α could act before antigen exposure, during migration of bone marrow–derived cells into nascent lymphoid follicles, establishing in a permissive fashion cellular interactions that allow subsequent antigen-dependent GC formation. LT-α is expressed by activated B lymphocytes (*20*), suggesting a possible mechanism by which the activated B cell itself could provide the focal stimulus for GC development.

GCs are also absent in mice and humans deficient in the TNF family members CD40 or the CD40 ligand (CD40L) (*21*). The absence of GCs in animals deficient in either CD40L or LT-α suggests either that signals through CD40 and TNFR-I are both required for the formation of GCs or that activation of these receptors is sequential in certain regulatory cells. In this regard, it is of interest that ligation of CD40 on B cells induces strong expression of LT-α (*20*). Thus, CD40L may act upstream of LT-α in the GC response. Finally, given the evolutionary relation between the TNFRs and CD40 and between LT-α and CD40L, it is possible that other members of this ligand and receptor family may also contribute to cellular organization in developing GCs. Specifically, although it is appealing to postulate that LT-α (presumably in its secreted homotrimeric form) regulates GC formation by binding directly to TNFR-I, it

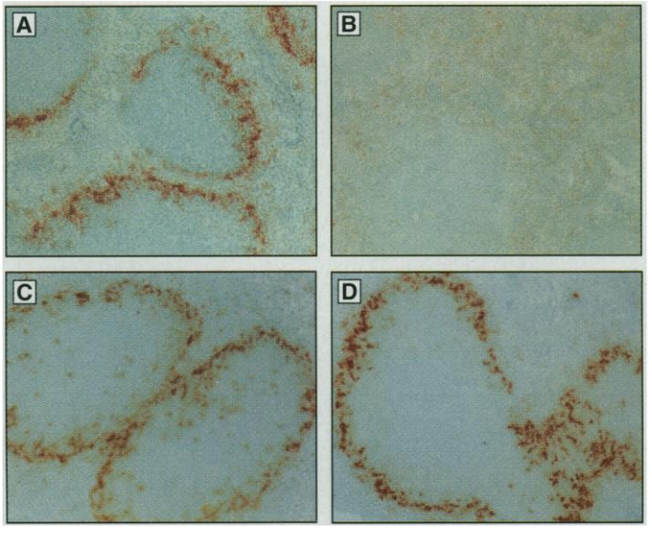

Fig. 2. MOMA-1 staining patterns of spleen sections from wild-type (**A**), LT-$\alpha^{-/-}$ (**B**), TNFR-I$^{-/-}$ (**C**), and TNFR-II$^{-/-}$ (**D**) mice. The staining pattern of TNFR-I$^{-/-}$ spleen sections is similar to that of sections from wild-type and TNFR-II$^{-/-}$ spleens. In contrast, LT-$\alpha^{-/-}$ spleen shows a disturbed pattern with near absence of MOMA-1 staining. Magnification, ×30.

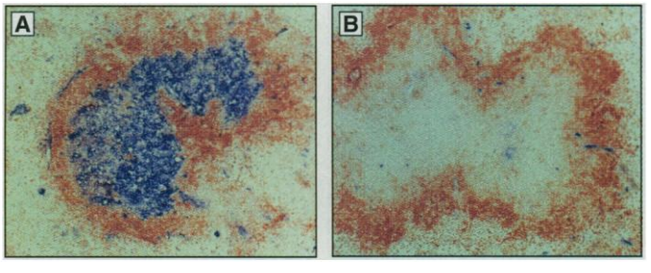

Fig. 3. Restoration of GC formation in LT-$\alpha^{-/-}$ mice by transplantation with normal bone marrow. (**A**) LT-$\alpha^{-/-}$ mice reconstituted with wild-type bone marrow showed restored formation of GCs after immunization with SRBCs. Staining is with PNA (blue) and antibody to IgD (brown). (**B**) Conversely, wild-type mice reconstituted with LT-$\alpha^{-/-}$ bone marrow showed no detectable development of GCs. Magnification, ×30.

should be acknowledged that TNF-α itself may contribute independently to the GC response.

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8. Mice were immunized intraperitoneally with 100 μl of phosphate-buffered saline containing 10% SRBCs (*22*). Ten days later, spleens were harvested, embedded in O.C.T. compound (Miles), and frozen in liquid nitrogen. Frozen tissue sections (6 to 10 μm thick) were fixed in cold acetone. Endogenous peroxidase was quenched with 0.2% H_2O_2 in methanol. Sections were stained by first incubating with PNA-biotin (Vector) and with rat antiserum produced to IgD (Southern Biotechnology). After washing, the sections were further incubated with streptavidin conjugated with alkaline phosphatase (AP) (Zymed) and rabbit antibody to rat IgG conjugated with horseradish peroxidase (HRP) (Southern Biotechnology). Color development for bound AP and HRP was with an AP reaction kit (Vector) and with diaminobenzidine. Sections were then counterstained with 1% methyl green.
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23. We thank J. Cyster for discussions regarding MOMA-1 staining, O. Kanagawa and R. Schreiber for critical reading of the manuscript, and B. Wright and G. Huang for assistance with histological evaluations. This work was supported in part by grants from NIH (D.D.C.) and the Monsanto Company (M.H.N.). D.D.C. is an investigator of the Howard Hughes Medical Institute.

8 November 1995; accepted 16 January 1996

HIV-1 Langerhans' Cell Tropism Associated with Heterosexual Transmission of HIV

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Heterosexual transmission by vaginal intercourse accounts for most transmission of human immunodeficiency virus–type 1 (HIV-1) in Africa and Asia but is less important in the HIV-1 epidemics of the United States and Western Europe. Epithelial Langerhans' cells (LCs) represent a possible source of initial cell contact for vaginal infection. Fifteen primary isolates of HIV-1 from U.S. homosexuals and 18 HIV-1 isolates from Thailand heterosexuals were evaluated for growth in LCs of U.S. origin. All the viruses from the Thai heterosexuals, which were subtype E, grew more efficiently in the LCs than any of the viruses from the U.S. homosexuals, which are subtype B. These results suggest that LC tropism is associated with the efficiency of heterosexual transmission of HIV.

In Thailand, India, and sub-Saharan Africa, it has been estimated that 90% of HIV-1 infections are acquired through heterosexual exposure (*1*, *2*). In contrast, most transmissions of HIV in the United States and Western Europe are associated with anal intercourse among homosexual men or injection drug use (IDU), with less than 10% of infections attributed to heterosexual contact (*3*). HIV-1 subtypes or clades have been classified according to HIV envelope and *gag* gene sequences (*4*). HIV-1 subtypes A through I have been described (*2*, *5*), and subtypes A through E have been characterized for geographical distribution, with A, C, and D dominating in sub-Saharan Africa, C dominating in India, and E dominating in Thailand (*2*, *5*). HIV-1 B is the dominant subtype reported thus far from the United States or Western Europe (*4*). HIV-1 B has also been isolated occasionally in Africa, Thailand, and India but is unusual there except in individuals infected through IDU (*2*, *6*). HIV-1 B is also the dominant subtype in the Caribbean and in South America, where heterosexual transmission is more common than in the United States but less common than in Africa or Thailand (*1*). Factors known to alter the efficiency of heterosexual transmission include other venereal diseases, condom use, male circumcision, and anal intercourse (*7*).

The uneven expansion of HIV-1 subtypes raises the possibility that some viruses have less potential for heterosexual transmission in comparison to others. Such enhanced susceptibility of individuals in particular risk groups has been corroborated in Thailand by comparison of heterosexual transmission of HIV-1 subtypes between couples (*8*). For heterosexual contact, Langerhans' cells (LCs) have been proposed as a possible primary target for HIV infection (*9*–*11*). These cells, which express CD4 on their membranes, are located on the surfaces of the oral and genital mucosa and are particularly abundant in the cervix, but are absent from rectal mucosa (*12*). To evaluate LC infectivity in relation to heterosexual transmission, we inoculated primary LC cultures with primary HIV-1 isolates from U.S.

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