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EFFECT OF TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-2 ON SPLEEN LYMPHOCYTE MIGRATION IN MOUSE SKIN

J. Sun, Z. Liu, Y. Bi, Z. Guo, T. Hua, Z. Ding

Department of Anatomy, Shandong Medical University, Jinan, People's Republic of China and Department of Biological Sciences (ZG), Kent University, Ohio USA

ABSTRACT

Tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2) are reported to enhance lymphocyte binding to endothelial cells in vitro. *We examined these two agents on lymphocyte migration* in vivo. *Spleen lymphocytes were radiolabeled with tritiated uridine (3H-UR) and then injected IV into mice. Each cytokine (TNF-α or IL-2) or both cytokines were then injected intradermally on the back of mice. The results demonstrated that TNF-α stimulates lymphocyte migration* in vivo *in dose-dependent fashion. Kinetic analysis demonstrated that migration with TNF-α started at 3h, peaked at 6h, followed by a gradual decline back to baseline at 24h. IL-2, on the other hand, was nearly inactive, and did not augment lymphocyte migration over and above that induced by TNF-α when both cytokines were injected together.*

During inflammation and recirculation, lymphocytes migrate into tissues by traversing the capillary endothelium, a process known as extravasation. The circulatory and migratory properties of the lymphocyte have evolved for efficient surveillance for infectious tissue pathogens and for rapid arrival at sites of injury and infection. Lymphocytes "patrol" the host for foreign antigen by recirculating from blood, through tissue, into lymph and back to blood. Lymphocyte migration is important for immune reaction. The initial step is lymphocyte adhesion to the blood capillary endothelium. During recirculation, lymphocytes exit the bloodstream by traversing the high endothelial venule (HEV) into lymphoid tissue such as lymph nodes and Peyer Patches (1). Lymphocyte migration into inflammatory sites is also thought to depend upon lymphocyte adhesion to the vascular endothelium, and these adhesion are mediated, at least in part, by adhesion receptors (ARS) on the surface of lymphocytes and endothelial cells. Experiments *in vivo* have demonstrated that lymphocytes bind to human umbilical vein endothelium stimulated with TNF-α (2). TNF-α can also increase the expression of ARS on the surface of endothelial cells (3). IL-2 has also been reported to have chemoattractant properties for lymphocytes (4-6) but the effects of TNF-α and IL-2 on lymphocyte migration *in vivo* remain unclear. Accordingly, we labeled spleen lymphocytes with 3H-UR and injected them IV into mice. The cytokines TNF-α and IL-2 were then injected intradermally alone or together and the migration of transference of lymphocytes into skin was examined.

MATERIALS AND METHODS

Animals and Reagents

Inbred BALB/C female mice, 7-9 weeks of age, were used in all experiments.

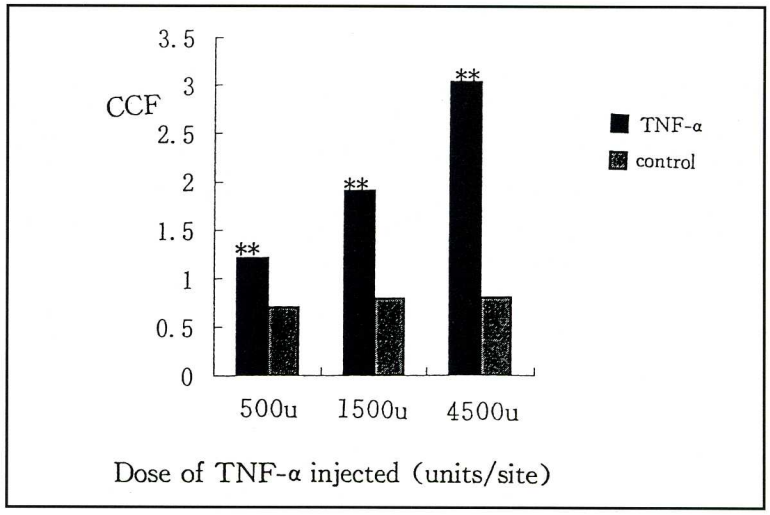

*Fig. 1. Accumulation of spleen lymphocyte migration into skin sites injected with varying doses of TNF-α (N=5 in each group). The control groups were injected with normal saline (N=5). **p<0.01; CCF=cell concentration factor.*

Recombinant human TNF-α (Sigma) and recombinant human IL-2 (Sigma) were the cytokines examined.

Isolation of Lymphocytes (7)

Mice were killed by cervical dislocation, and the spleen was immediately excised. Single cell suspensions were prepared by rubbing the spleen on a 30-buffered ammonium chloride solution (0.18 M of NH_4Cl in 0.017 M Tris-HCl, pH 7.2) for 3 min at room temperature. After washing twice with 10 ml of PBS, the cells were resuspended in DMEM containing antibiotics (10u of penicillin and 25 ug of gentamicin per ml). The cells were incubated in tissue culture plate for 60 min, at 37°C to allow the macrophages to adhere. Non-adhesion cells were removed and washed once with the same culture medium. Cell viability was more than 90% as determined by eosin Y exclusion.

Cell Labeling

Lymphocytes were labeled with tritiated uridine (3H-UR). These cells were briefly suspended in RPMI 1640 medium. 3H-UR was added to the medium until the final concentration was $3.4x10^5$ BQ/ml. After 6h, cells were washed and resuspended for IV injection. Each mouse was injected with $1x10^7$ lymphocytes. The viability of the cells was >95% as determined by trypan blue exclusion.

Experimental Design

Mice anesthetized with ether were injected IV with 3H-UR labeled lymphocytes. Immediately thereafter, the hair on the back of the mouse was shaved, and TNF-α and IL-2 were injected with 500u, 1500u, 4500u, respectively, into two sites. An additional group was injected with normal saline (NS). The mice were sacrificed at 3, 6, 9, 12, 15, 18, 21, and 24h (N=5, for each group) after injection. The skin on the back of the mice was cut off, excess blood in the superficial veins was squeezed out and then the injected areas were punched out with a leather punch. The skin tissue of injected region was put into a mixture of 0.3ml HCOOL and H_2O_2 at 70-80°C for 60 min. Through emulsion counting, the counts per minute (CPM) of 3H-UR was examined.

Analysis of Data

The cell concentration factor (CCF) was determined by calculation where CCF=cpm/g

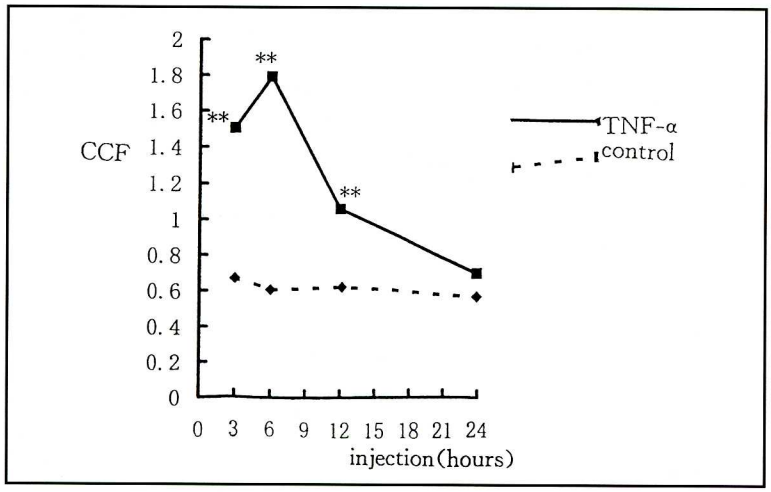

*Fig. 2. Kinetics of lymphocyte migration in response to TNF-α. Lymphocyte migration enhancement was seen at 3h, peaked at 6h, and gradually declined approaching control levels at 24h. **p<0.01; CCF=cell concentration factor.*

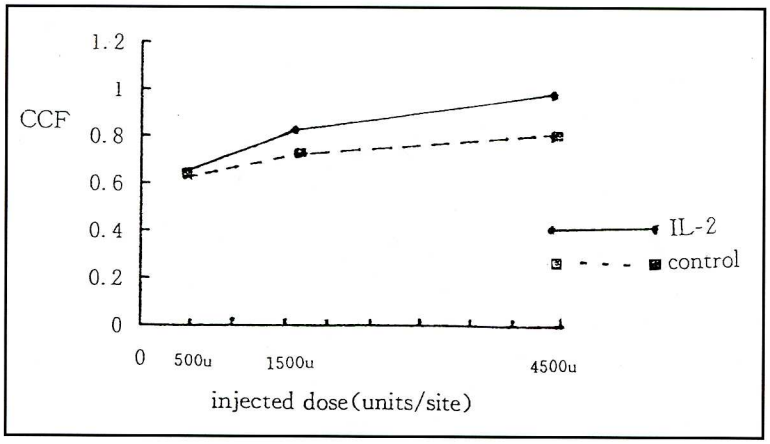

Fig. 3. Accumulation of lymphocyte in the skin sites after injection with varying doses of IL-2 (500u, 1500u, and 4500u, respectively) (N=5). The control was injected with normal saline (N=5). Note there was no effect on lymphocyte migration; CCF=cell concentration factor.

skin tissue punched/total cpm injected/body wt (g). A student t-test was used for statistical analysis.

RESULTS

Lymphocyte Recruitment by TNF-α

Fig. 1 shows that injection of TNF-α recruited a large number of labeled cells into skin in dose dependent fashion. Thus, 500u of TNF-α induced a single-fold increase of lymphocytes, 2-fold at 1500u and 3-fold at 4500u compared with control (normal saline).

Kinetics of Lymphocyte Migration in Response to TNF-α

Fig. 2 shows that 4500u TNF-α induced a rapid increase in lymphocyte migration into the skin before 3h, peaked at 6h and gradually declined thereafter. By 24h, lymphocyte migration into the skin approached control levels.

Effect of IL-2 on Lymphocyte Migration

IL-2 was administered to mice in doses of 500u-4500u but no effect on lymphocyte migration into the skin was detected *(Fig. 3)*.

Interaction of TNF-α and IL-2 on Lymphocyte Recruitment

Because TNF-α and IL-2 interact in several biological systems, the effect of a

TABLE 1
Lymphocyte Entry Into Skin Sites Induced by Mixtures of TNF-α and IL-2

Injected stimulus	N	Dose (units)	CCF
TNF-α	5	1500	1.90±0.36
IL-2	5	1500	0.85±0.22
TNF-α + IL-2	5	1500+1500	$1.95\pm0.29_{a,b}$

a) Significances comparing mixture cytokine with TNF-α and IL-2 alone was $p>0.05$ and $p<0.01$, respectively.
b) The cell concentration factor (CCF) of mixture cytokine was 71% of the sum of TNF-α and IL-2 alone.

combination of these two cytokines on lymphocyte recruitment was investigated. *Table 1* shows that a combination of TNF-α and IL-2 stimulated a response which was less than the sum of either agent alone. In short, IL-2 did not potentiate lymphocyte migration induced by TNF-α.

DISCUSSION

Lymphocyte migration from the blood microcirculation into tissues is a complex process with important implications for both lymphocyte recirculation and for inflammation/infection. During lymphocyte extravasation the cells undergo at least four steps: 1) adhesion to the blood capillary; 2) migration to the endothelial junction; 3) migration between adjacent endothelial cells; 4) penetration of the basement membrane. Many studies have suggested that lymphocyte migration into tissues is controlled, at least in part, by the adhesion molecule on the surface of the lymphocyte and endothelium. Lymphocytes also bind *in vitro* to endothelial cells stimulated with TNF-α (2). Our studies show that TNF-α stimulates lymphocyte migration in mice *in vivo* although the exact mechanism remains unclear. TNF-α may promote greater adhesion molecule expression on endothelia. Intercellular adhesion molecule-1 (ICAM-1) is expressed on endothelia. When endothelium is stimulated with TNF-α, the expression level of ICAM-1 is greatly increased. Another adhesion molecule, vascular cell adhesion molecule (VCAM-1) is also induced on endothelial surfaces (9).

Selective lymphocyte-endothelial recognition is a function of lymphocyte and activation status as well as the tissue specific expression of endothelial cell ligand. The expression of adhesion molecule on vascular endothelial cells and non-vascular skin cells is dependent upon the action of soluble mediators or cytokines. In the skin, keratinocytes and T-lymphocytes secrete a large number of cytokines that are capable of amplifying the inflammatory response. One of the critical mediators is TNF-α (10). It is likely that inducible endothelial cell ligands including VCAM-1 are up-regulated with inflammation. The data from immunohistochemical studies of adhesion molecule expression in human cutaneous reactions suggests that ICAM-1, VCAM-1, and other adhesion molecules are expressed *in vivo* (11).

TNF-α influences lymphocyte migration through other pathways. Several studies suggest that TNF-α interacts with compo-

nents of the basement membrane, such as fibronectin (FN) and laminin (LN) (12,13). TNF-α—LN complexes also enhance the binding of lymphocyte to the basement membrane. Accordingly, TNF-α may direct cell migration and recruitment of lymphocytes to inflammatory tissue by binding to components of the basement membrane. Whereas the process of TNF-α induced lymphocyte migration is complex, the present kinetic study shows that lymphocyte recruitment by TNF-α was sustained for nearly 24h, with a peak at 6h, and then gradually declining.

IL-2 has multiple immune enhancing activities related to its ability to promote the proliferation and the expression of effector function of human lymphocytes. Our results demonstrated, however, that IL-2 was unable to stimulate lymphocyte migration to skin. Some experiments favor that IL-2 activates lymphocyte locomotion (6), but others demonstrated that IL-2 did not promote lymphocyte binding to endothelium (14). Most likely, locomotion is the initial event of lymphocyte migration. Whereas IL-2 has induced lymphocyte locomotion *in vitro*, it did not induce lymphocyte migration to skin *in vivo*.

We also examined the interaction of TNF-α and IL-2 on lymphocyte migration. Whereas TNF-α stimulated spleen lymphocyte migration in mice *in vivo*, IL-2 was inactive and did not potentiate lymphocyte migration over and above that induced by TNF-α alone. In effect, there was no synergy of TNF-α and IL-2 in inducing lymphocyte migration into the skin of mice.

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Zhiyu Liu, M.D.
No. 44 Wei Hua Xi Road
Shandong Medical University
Department of Anatomy
Jinan, Shandong 250012
PEOPLE'S REPUBLIC OF CHINA