Effect of Tumor Necrosis Factor-α and Interfereon-α on Spleen Lymphocyte Migration in Mouse Skin

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Abstract **Objective** To examine the effect of lymphocyte migration in vivo. **Methods** Spleen lymphocytes were radiolabeled with tritiated uridine (3H-UR) and then were injected IV into mice. Each cytokine (TNF-α or IFN-α) or both cytokines were then injected intradermally on the back of mice. 20 hours later, the injected areas were punched out, through emulsion counting, the counts per minute (CPM) of 3H was examined, and the cell concentration factor (CCF) was determined by calculation. **Results** The CCF of TNF-α and IFN-α were more than that of control groups. The CCF of mixture cytokine was 137% of the sum of TNF-α and IFN-α alone. **Conclusion** TNF-α could stimulate lymphocytes migration in vivo in dose-dependent fashion. IFN-α, might also promote lymphocytes migration in mouse skin. Besides, IFN-α could augment lymphocyte migration over and above that induced by TNF-α when both cytokines were injected together.

Key Words Tumor necrosis factor-α; Interferon-α; Lymphocyte; Migration

Most lymphocytes continuously recirculate between the blood and lymphoid tissues. Lymphocytes extravasate from the blood mainly through a specialized endothelium of postcapillary high endothelial venules (HEVs)[1]. At least four functionally distinct lymphocyte-endothelial cell recognition systems exist that control lymphocyte traffic to peripheral lymph nodes, to mucosal sites, to inflamed synovium, and the inflamed skin[2]. The first step in lymphocyte extravasation, binding of lymphocytes to endothelial cells, is critically controlled by interactions between homing-associated molecules on the lymphocyte surface and their ligands on HEVs[3-4]. These molecules that confer the specificity and stability of the adhesive interactions include members of several distinct adhesion receptor families. For example, on lymphocytes L-selectin mediates lymphocyte binding to vascular endothelium in peripheral lymph nodes and integrin, very late activation antigen 4 (VLA-4), participates in homing at mucosal lymphatic sites. Besides, CD44 and lymphocyte function-associated antigen 1 (LFA-1) serve as general adhesion molecules strengthening lymphocyte-HEV interaction in a non-organ-specific fashion.

Experiments in vivo have demonstrated that lymphocytes bind to human umbilical vein endothelium stimulated with TNF-α[5]. TNF-α can also increase the expression of ARS on the surface of endothelial cells in vitro[6]. IFN-α has also been reported to have chemoattractant properties for lymphocytes[7-8], but the effects of TNF-α and IFN-α on lymphocyte migration in vivo remains unclear. Accordingly, we labeled spleen lymphocytes with 3H-UR and injected them IV into mice. The cytokines TNF-α and IFN-α were then injected intradermally alone or together and the migration of transference of lymphocytes into skin was examined.

MATERIALS AND METHODS

Animal and Reagents

Inbred BALB/C female mice, 7-9 weeks of age, were used in all experiments. Recombinant human TNF-α (Sigma), recombinant human IFN-α (Sigma) were the cytokines examined.

Isolation of Lymphocytes

Mice were killed by cervical dislocation and the spleen was immediately excised. Single cell suspension 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 re-suspended 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℃ to allow the macrophages to adhere. Non-adhesion cells

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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 3.4×10^5 BQ/ml. After 6h, cells were washed and resuspended for IV injection. Each mouse was injected with 1×10^7 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 was injected with 500u, 1500u, 4500u (N=5, for each group), IFN-α was injected with 1000u, 2000, 4000u (N=5, for each group), mixtures of TNF-α (1500u) and IFN-α (2000u) (N=5), into two sites respectively. An additional group was injected with normal saline (NS). The mice were sacrificed at 20h 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℃ for 60 min. Through emulsion counting, the counts per minute (CPM) of ^{3}H-UR was examined.

Analysis of Data

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

Effect of IFN-α on Lymphocyte Migration

IFN-α was administered to mice in doses of 1000u, 2000u and 4000u, following the increase of doses, more labeled lymphocytes were recruited into skin (Fig.2).

Interaction of TNF-α and IFN-α on Lymphocyte Recruitment

Because TNF-α and IFN-α interact in several biological systems, the effect of a combination of these two cytokines on lymphocyte recruitment was investigated. Table 1 showed that a combination of TNF-α and IFN-α stimulated a response which was more than the sum of either agent alone. In short, IFN-α could potentiate lymphocyte migration induced by TNF-α.

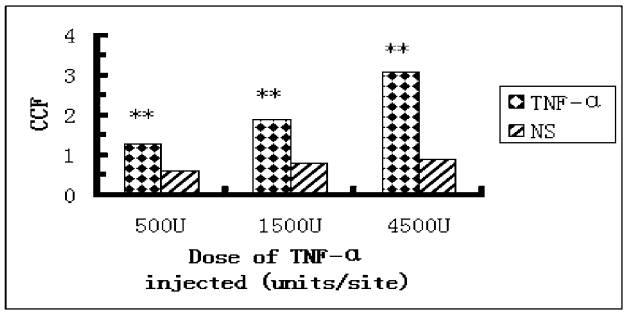

Fig.1 Accumulation of spleen lymphocyte migration into skin sites injected with varying doses of TNF-α (N=5 in each group). The control groups were injected with normal saline (N=5). **p<0.01; CCF=cell concentration factor.

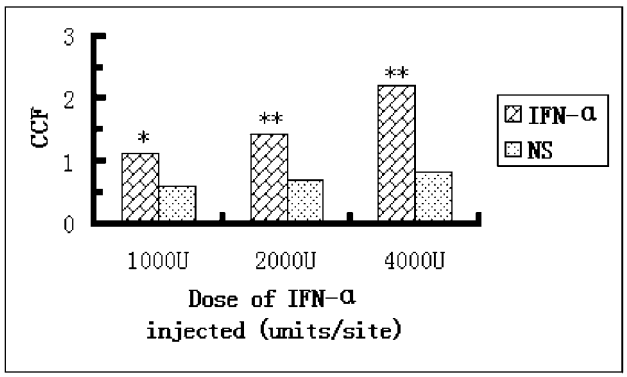

Fig.2 Accumulation of spleen lymphocyte migration into skin sites injected with varying doses of IFN-α (N=5 in each group). The control groups were injected with normal saline (N=5). *p<0.05, **p<0.01; CCF=cell concentration factor.

Table 1 Lymphocyte entry into skin sites induced by mixtures of TNF-α and IFN-α

Injected stimulus	N	Dose (units)	CCF
TNF-α	5	1500	1.90±0.36
IFN-α	5	2000	1.43±0.29
TNF-α + IFN-α	5	1500+2000	$4.56\pm0.39_{a,b}$

a) Significances comparing mixture cytokine with TNF-α and IFN-α alone was both p<0.01.

b) The cell concentration factor (CCF) of mixture cytokine was 137% of the sum of TNF-α and IFN-α alone.

DISCUSSION

The exquisite tissue-selective homing of lymphocytes has long been appreciated as central for the control of systemic immune responses. Naive lymphocytes display a relatively homogeneous migration behavior, recirculating through secondary lymphoid tissues, including lymph nodes, Peyer's patches, tonsils, and spleen, to encounter their cognate antigen. Following antigen encounter and recognition in an appropriate microenvironment, lymphocytes differentiate into effector/memory cells and acquire the ability to access extralymphoid immune effector sites where they are most likely to reencounter their specific antigen[9]. 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. 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]. Our studies showed that TNF-α stimulated 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 [11]. 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 suggested that ICAM-1, VCAM-1, and other adhesion molecules were expressed in vivo[12].

TNF-α may influences lymphocyte migration through other pathways. Several studies suggested that TNF-α interacts with components of the basement membrane, such as fibronectin (FN) and laminin (LN)[13, 14]. 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, it could affect many steps during migration.

IFN-α has antiproliferative, antiviral, and immunomodulatory functions. In addition, IFN-α has anticancer activity in vivo[15]. In our experiments, IFN-α could promote lymphocyte migration. The cutaneous lymphocyte-associated antigen (CLA) defines a subset of circulating memory T cells that selectively localize in cutaneous sites, this process is mediated in part by the interaction of CLA with its vascular ligand E-selectin[16]. IFN may up-regulate the expression of E-selectin on endothelium. During inflammation in skin, many inflammatory cells may secrete IFN-α. But more molecular basis of IFN-α action needs to be understood.

We also examined the interaction of TNF-α and IFN-α on lymphocyte migration. TNF-α stimulated spleen lymphocyte migration in mice in vivo, similarly, IFN-α was active and could potentiate lymphocyte migration over and above that induced by TNF-α alone. In effect, there was a synergy of TNF-α and IFN-α in inducing lymphocyte migration into the skin of mice.

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