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THE ROLE OF VCAM-1/VLA-4 IN THE ACTIVATION OF ALLOGENIC T CELLS BY MURINE MACROPHAGES*

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Vascular cell adhesion molecule 1 (VCAM-1) is a member of immunoglobulin superfamily. The principal ligand for VCAM-1 is integrin α4β1/VLA-4 (very late antigen 4). It was reported that VCAM-1 was expressed on macrophages and dendritic cells, but little is known about its function on these professional antigen presenting cells (APC). The present study was performed to investigate the expression of VCAM-1 on macrophages and the role of VCAM-1/VLA-4 in the activation of allogenic T cells by murine macrophages. We analyzed VCAM-1 expression on peritoneal macrophages and macrophage cell line J774A.1 by fluorescence-activated cell sorting (FACS). Using neutralizing antibodies, we further analyzed the role of VCAM-1/VLA-4 interaction in macrophage and allogenic T cell mixed lymphocyte reaction (MLR). We found that VCAM-1 was constitutively expressed on macrophages and its expression level was upregulated by soluble tumor associated antigen (freeze-thaw lysates of FBL-3 leukemia cells) and TNF-α. In MLR assays, we observed that blocking VCAM-1/VLA-4 interaction with anti-VCAM-1 or anti-VLA-4 mAbs caused significant inhibition of the proliferative response and IL-2 production. These results suggest that VCAM-1on macrophages not only facilitates the cell-to-cell contact through adhesive interaction but also plays a role in the costimulation of T cells via its interaction with VLA-4 on the T cells.

Key words: VCAM-1, VLA-4, Macrophages, T cells, Costimulation.

Antigen presentation and specific T cell activation play important roles in the host immune responses against disease. At present, studies on the immunotherapy and gene therapy based on the activation or inhibition of antigen-presenting cells (APC) including dendritic cells, macrophages attract more attention.[1,2] It is important to elucidate the mechanisms by which APCs present antigen to T cells and activate T cells so as to outline new approaches to the treatment of disease.

Macrophage (Mϕ) is a kind of professional APC capable of priming T lymphocytes response to foreign antigen. In addition to the recognition of the MHC/antigen complex by the TCR, initiation of an effective T lymphocyte response requires the delivery of a second or costimulatory signal. Cell surface ligand receptor systems such as ICAM-1/LFA-1, LFA-3/CD2, B7-BB1/CD28 have been implicated in regulating T cell activation and proliferation. Absence of these costimulatory signals leads to anergy of T cell. Vascular cell adhesion molecule 1(VCAM-1) is a member of immunoglobulin superfamily (IgSF). Because of its inducible expression on activated endothelial or epithelial cells by inflammatory factor including TNF-alpha, IL-1 beta, LPS, it was also known as inducible cellular adhesion molecule (INCAM). Now, it was designated to be CD106.

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VCAM-1 interacted with integrin family members, alpha 4 beta 1 and alpha 4 beta 7. The principal ligand for VCAM-1 is alpha 4 beta 1 (CD49d/CD29)/Very Late Antigen-4 (VLA-4), which is expressed on lymphocytes, monocytes, and some granulocytes but is absent from polymorphonuclear neutrophils. The VCAM-1/VLA-4 interactions play a role in cell trafficking, adhesion, development and involves in pathological process, such as inflammation, malignant metastasis, autoimmune diseases, parasite infection.[3] In recent years, using a soluble VCAM-1 fusion protein with receptor globulin (Rg), Damle et al.[4] identified that VCAM-1/VLA-4 could function as costimulation signal in T cell activation. Using immunoperoxidase techniques, Rice et al[5] found that VCAM-1 was expressed on the macrophages and dendritic cells. However, the properties and function of VCAM-1's expression on these professional APCs are not known. To ascertain the role of VCAM-1 on macrophages, in the present study we preliminarily analyzed the expression of VCAM-1 on macrophages and the role of VCAM-1/VLA-4 in the activation of allogenic T cells by murine macrophages.

MATERIALS AND METHODS

Reagents

Culture medium RPMI1640 and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (USA). Monoclonal rat IgG2b antibody to murine VCAM-1 (M/K1.9), VLA-4 α chain (PS/2), F4/80, Ia (B21-2) and Control, subclass-matched IgG_{2b} antibody 7D2-1.4.1.5 were produced from hybridomas obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and purified from ascites by protein G affinity chromatography. FITC-conjugated goat anti-rat immunoglobulin antibody and rhTNF-α were purchased from Gibco (USA). Mini-magnetic bead (<50 nm) conjugated goat anti-rat IgG and MiniMACS (Minimagnetic cell sorter) column were the products of Militenyi Biotec GmbH in German. Mouse IL-2 ELISA kit was obtained from Endogen (USA).

Mice and Cell Lines

Female BALB/c and C57BL/6 mice, 6–8 weeks old, were purchased from Sino-British SPIR/BK Animal Corp. in Shanghai. FBL-3 cell line was derived from Friend Leukemia Virus induced C57BL/6 leukemia cell line; J774A.1 (ATCC, TIB-67), a monocyte-macrophage tumor cell line, was cultured with DMEM plus 10% fetal bovine serum.

FBL-3 Tumor Cells Lysates Preparations

After routine propagation, FBL-3 tumor cells were washed twice with phosphate-buffered saline (PBS, pH 7.2) and resuspended at 1×10^7 cells/ml in PBS. Then FBL-3 cells were disrupted by three freeze-thaw cycles and centrifuged at 600×g for 20 min. The supernatant was collected and spun again at 13,000×g for 1 h. The remaining supernatant was used as a source of soluble tumor associated antigen (TAA).

Preparation of Peritoneal Macrophages

Peritoneal macrophages were collected from normal mice injected 2 ml of 3% isothiocyanate broth medium (Sigma) ten days before. Peritoneal lavage was performed using 10 ml of ice cold HBSS containing 10 μ/ml heparin without calcium or magnesium. Macrophages in supplemented RPMI-1640 were plated in 100-mm diameter plastic Petri dishes then incubated for 3 h at 37℃in an atmosphere with 5% CO_2 and then washed three times with 10 ml HBSS to remove nonadherent cells. The adherent cells containing high purified macrophages were harvested using a rubber policeman and resuspended in complete RPMI-1640 medium for further use.

Flow Cytometry

Macrophage populations (5×10^5) were first incubated with 1ug of mAb against VCAM-1 (M/K1.9) for 45–60 min at 4℃, washed two times with PBS supplemented with 1% BSA and 0.02% NaN_3 (washing buffer), and then treated with Fluorescein isothiocyanate (FITC)-conjugated goat anti-rat immunoglobulin antibody for 30–45 min at 4℃. After washing three times with washing buffer, cells were fixed in 0.1ml of 1% polyformaldehyde (PFA) in PBS for 15 min on ice and then analyzed on a FACScan (Becton-Dickinson, Immunocytometry Systems, Mountain View, CA). A nonbinding mAb 7D2-1.4.1.5 was used as the negative control antibody.

T Cell Preparation and Mixed Lymphocyte

Reaction (MLR)

Splenic cell suspensions from normal mice were depleted of red blood cells with ammonium chloride-potassium lysing buffer, passed through a nylon wool (Gibco) to deplete B cells. The T cell-enriched population was further incubated with anti-Ia mAb (B21-2) 45 min at 4℃, washed and then treated with Mini-magnetic bead-conjugated goat anti-rat IgG for 15 min at 10℃, subsequently depleted of Ia^+ cells by passing through MiniMACS column. The negatively selected populations were >98% enriched for T cell as assessed by flow cytometry, and viable cell counts were >90% by trypan blue exclusion. For the primary MLR, responder T cells (1×10^5) were mixed with graded (R/S) 3,000 rad-irradiated stimulator macrophages in 96-well flat-bottom tissue culture plates and cultured for 72 h at 37℃ in a 5% CO_2 and 95% air atmosphere. The cultures were then pulsed with 1 uCi [^{3}H]thymidine (Shanghai Nuclear Energy Institute) for an additional 18 h incubation. Incorporation of ^{3}H-TdR is measured by liquid scintillation counting (Wallac 1409). Results are mean cpm from triplicate cultures and expressed as cpm± SD.

IL-2 Assay

Supernatant levels of IL-2 were determined by ELISA according to standard procedures.

Statistical Analysis

Data were analyzed with the Student's *t*-test (one tail).

RESULTS

Expression of VCAM-1 on the Macrophages

As shown in Figure 1, VCAM-1 was fundamentally expressed on both peritoneal macrophages and macrophage cell line J774A.1. The expression level was enhanced upon the overnight stimulation with 0.5ml FBL-3 tumor cells lysates or TNF-α (10 ng/ml). The results of the two types of macrophages were so coincident that we suggest that VCAM-1 is constitutively expressed on the macrophages and its expression level could be upregulated by soluble tumor associated antigen and cytokine such as TNF-α.

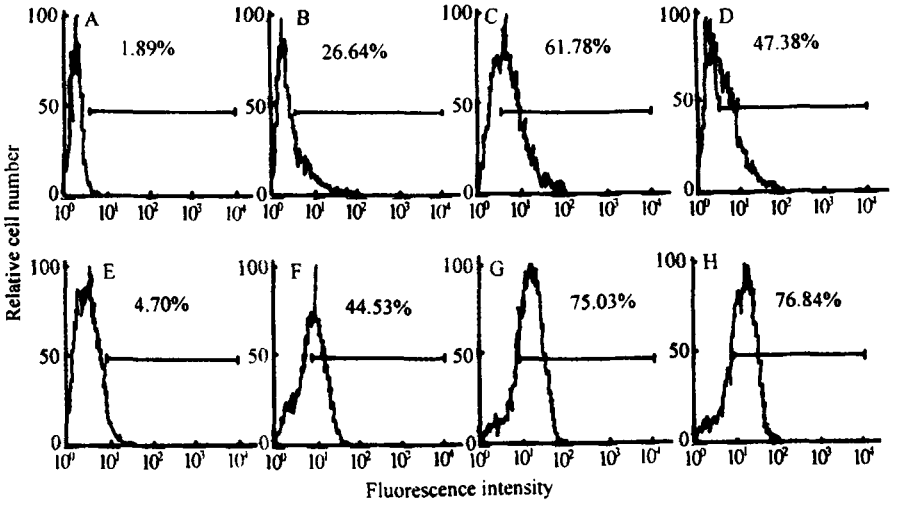

Fig. 1. FACS analysis for expression of VCAM-1on the peritoneal macrophages and macrophage cell line J774A.1. A, E: control; B, F: normal; C, G: TNF-α treated; D, H: FBL-3 lysates treated.

The Role of VCAM-1/VLA-4 in the Activation of Allogenic T Cells by Murine Macrophages

In the MLR responses (R/S=4:1), we found that treatment before mixed cultures with anti-VCAM-1 mAb (M/K1.9, 10 μg/ml) or anti-VLA-4 mAb (PS/2, 10 μg/ml) respectively caused a significant attenuation of T cell proliferation (Figure 2). The production of

IL-2 during MLR plays an important role in the allospecific proliferation of T lymphocytes. Using ELISA kit, we assayed the IL-2 level in supernatants from peritoneal macrophages (5×10^5) and allogenic T cells (2×10^6) mixed cultures in the presence or absence of neutralizing antibodies in 12-well plate for 4 days. As shown in Figure 3, IL-2 production was ranged from 128 to 216 pg/ml in the absence of neutralizing antibodies, while ranged from 64 to 125 pg/ml in the presence of M/K1.9 or PS/2; treatment of MLR cultures with anti-VCAM-1 mAb or anti-VLA-4 mAb resulted in 40% reduction in IL-2 production. There was no distinct difference between the blocking effects of M/K1.9 and PS/2. Control antibody 7D2-1.4.1.5 failed to alter the proliferative response and IL-2 secretion during MLR. These observations established that the interaction of VCAM-1 with VLA-4 played an important role in the proliferation of T cells and IL-2 production in the MLR responses.

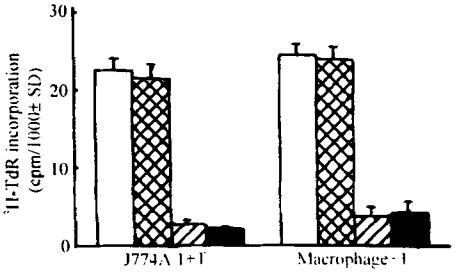

Fig. 2. Inhibition of T cell proliferation in MLR by anti-VCM-1 mAb (M/K1.9) and anti-VLA-4 mAb (PS/2).

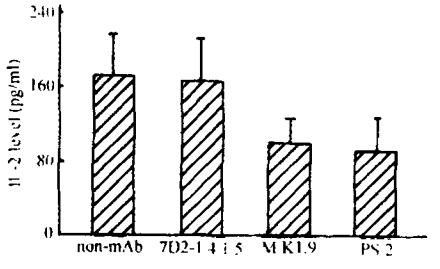

Fig. 3. Inhibition of IL-2 production in MLR by anti-VCAM-1 mAb (M/K1.9) and anti-VLA-4 mAb (PS/2).

DISCUSSION

VCAM-1 was first identified by Osborn et al,[6] who utilized its inducible expression on human umbilical cord vein endothelial cells and cloned it by subtractive hybridization. Because of its inducibility, VCAM-1 was also named INCAM. Previous studies have demonstrated that VCAM-1 was constitutively expressed on many types of cells, such as fibroblast, bone marrow stromal cell, myoblast etc.[7] VCAM-1was also expressed on macrophages, but what we knew was restricted in the study of immunohistochemistry,[5] and little is known about the effects of antigens and cytokines on its expression on APCs. We observed by FACS that VCAM-1 was constitutively expressed on macrophages and was upregulated by antigen or TNF-α. These suggest that VCAM-1 play an important role in macrophage function.

The study by Damle et al[3] indicated that VCAM-1/VLA-4 interaction can deliver costimulation signal to T cell activation and that costimulation via both VCAM-1 and T cell receptor (TCR) was sufficient for activation of IL-2 production and T cell proliferation. Lukacs et al.[8] found VCAM-1 have important implications during human peripheral blood mononuclear cells allogenic immune responses for the activation and proliferation of T lymphocytes as well as cytokine production. Our results showed the blocking effects of anti-VCAM-1 mAb or anti-VLA-4 mAb were similar in the inhibition of proliferation of T cells. These implicated indirectly that VCAM-1/VLA-4 involves in the costimulation of T cells. As macrophages represent one major type of the professional APC and VLA-4 is widely distributed and exists on the surfaces of leukocytes except neutrophils, we suggest that the regulatable expression of VCAM-1 on macrophages is critical in immune surveillance and in initiation of T cell immune response through costimulation.

In our study, macrophages of primary cultures as well as of established cell line were used as *in vitro* model system. J774A.1 cell line was adapted from a monocyte-macrophage tumor which arose in a female BALB/c mouse in 1968. There is little difference between this cell line and normal macrophage except in proliferational potency. Our results also confirmed that.

In the present study, the antigen we used was freeze-thaw lysates of FBL-3 tumor cells, which caused the upregulation of VCAM-1 expression. This indicated that tumor associated antigen was able to prime macrophage and increase the ability of macrophage to present antigen, hence trigger T cell immune responses.

In conclusion, these results suggest that the expression of VCAM-1 on macrophages, which serve as immune effector cells and antigen presenting cells, not only allows for increased cell-to-cell contact through adhesive interaction but also plays a role in the costimulation of T cells via its interaction with VLA-4.

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