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*J Immunol* 1999; 163:1619-1627; ;
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Evidence for the Involvement of CD44 in Endothelial Cell Injury and Induction of Vascular Leak Syndrome by IL-2

Asimah Q. Rafi-Janajreh,* Dawei Chen,† Rudolf Schmits,‡ Tak W. Mak,§ Randolph L. Grayson,† D. Phillip Sponenberg,‡ Mitzi Nagarkatti,§ and Prakash S. Nagarkatti‡*

At sites of chronic inflammation seen during infections, autoimmunity, graft-vs-host response, and cytokine therapy, endothelial cell injury is known to occur, the exact mechanism of which is unknown. In the current study we used IL-2-induced vascular leak syndrome (VLS) as a model to investigate whether cytotoxic lymphocytes use CD44 in mediating endothelial cell injury. Administration of IL-2 to wild-type mice triggered significant VLS in the lungs and liver. In contrast, in CD44 knockout (KO) mice, IL-2-induced VLS was markedly reduced in the lungs and liver. IL-2-treated wild-type and CD44 KO mice had similar levels of perivascular infiltration with lymphocytes in the lungs and liver. This suggested that the decrease in VLS seen in CD44 KO mice was not due to the inability of lymphocytes to migrate to these organs. Ultrastructural studies demonstrated extensive endothelial cell damage in the lungs and liver of IL-2-treated wild-type, but not CD44 KO, mice. Moreover, CD44-KO mice exhibited a marked decrease in IL-2-induced lymphokine-activated killer cell activity. The induction of VLS was dependent on the expression of CD44 on immune cells rather than endothelial cells because adoptive transfer of CD44+, but not CD44− spleen cells along with IL-2 into CD44 KO mice triggered VLS. The IL-2-induced VLS was blocked by administration of F(ab′)2 of Abs against CD44. The current study demonstrates that CD44 plays a key role in endothelial cell injury. Blocking CD44 in vivo may offer a novel therapeutic approach to prevent endothelial cell injury by cytotoxic lymphocytes in a variety of clinical disease models. The Journal of Immunology, 1999, 163: 1619–1627.

In a number of disease models, including infections, autoimmunity, transplantation, and graft-vs-host disease, severe damage to the endothelial cells leading to toxicity has been known to occur (1–5). However, the exact mechanism of such endothelial cell damage is not clear. Furthermore, IL-2 therapy has been shown to be effective in the treatment of certain types of cancer, although its use is limited by toxicity resulting from endothelial cell damage and capillary leak (6–8). Several cytokines, including those used as hemopoietic growth factors, have been shown to trigger toxicity resulting in increased capillary leak, also known as the vascular leak syndrome (VLS) (1) (7). It has been widely speculated that immune cells, particularly the cytotoxic lymphocytes, may play an important role in endothelial cell damage.

CD44 is a family of cell surface glycoproteins with proposed functions in extracellular matrix (ECM) binding, cell migration, lymphopoiesis, and lymphocyte homing (9). CD44 molecules are produced by alternate splicing of multiple exons of a single gene and by different posttranslational modifications in different cell types (9). The principal ligand of CD44 has been identified as hyaluronic acid (HA), a major component of the ECM.

We and others have shown that activated CTL, NK/lymphokine-activated killer (LAK) (4) cells, and cytotoxic double-negative T cells, express increased levels of CD44 and mediate efficient lysis of target cells when activated through CD44 (10, 11). Inasmuch as CD44 also plays a major role in the lymphocyte adhesion to the endothelial cells, we have hypothesized that dysregulation in the interaction between cytolytic lymphocytes expressing CD44 and endothelial cells bearing the appropriate ligand could lead to endothelial cell injury and VLS (10, 12). To this end, we have shown that IL-2-induced VLS is markedly decreased in mice deficient in perforin and Fas ligand, thereby suggesting the involvement of cytotoxic lymphocytes in VLS (12). Also, treatment with IL-2 caused a significant increase in the expression of CD44, migration and perivascular infiltration of lymphocytes in various organs, and endothelial cell damage (12).

In the current study we further tested the hypothesis that CD44 is directly involved in the injury to the endothelial cells caused by CTL and LAK cells during IL-2-induced VLS. To this effect we used CD44 knockout (KO) mice and observed that such mice exhibit markedly diminished VLS following IL-2-treatment. Our data also suggest that blocking CD44 helps in reducing the IL-2-induced VLS; therefore, such an approach may serve as a useful tool to prevent the endothelial cell damage seen in a variety of clinical disorders.

Materials and Methods

Mice

Adult female C57BL/6 mice (CD44−, wild type) were purchased from the National Institutes of Health (Bethesda, MD). CD44 KO mice with C57BL/6 background were generated at Amgen Institute (Toronto, Canada), bred in the animal facilities at Virginia Tech, and screened for the CD44 deletion. The phenotype of these mice has been described previously (13).
ROLE OF CD44 IN VASCULAR LEAK SYNDROME

Cell lines

P815, a mastocytoma resistant to NK cells, was maintained in vitro by serial passages in tissue culture medium, RPMI 1640 containing 10% FCS, as previously described (12).

Abs and reagents

Monoclonal MEL-14 (lymphocyte homing receptor; rat IgG) and anti-LFA-1 (M17/4; rat IgG) Abs were grown in vitro as previously described (12). The FITC-anti-CD3, PE-anti-CD44, PE-anti-CD8, and FITC-anti-CD4 mAbs were purchased from PharMingen (San Diego, CA). FITC-conjugated F(ab')2 of goat anti-Syrian hamster IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). F(ab')2 of anti-CD44 (IM7) mAbs were prepared by treatment with pepsin and passing the fragments over a protein A column (Pierce, Rockford, IL) (10). Evan’s blue dye was obtained from Sigma (St. Louis, MO).

Interleukins

Recombinant IL-2 was provided by Hoffmann-La Roche (Nutley, NJ) and by Dr. C. Reynolds (National Institutes of Health, Bethesda, MD).

Detection of surface molecules using immunofluorescence analysis

Splenic T cells and LN cells were analyzed for LFA-1 and other adhesion molecule expression using flow cytometry. For LFA-1 detection, the cells were incubated with anti-LFA-1 Abs for 30 min on ice followed by washing three times. After washing, FITC-conjugated secondary Ab was added to detect the presence of LFA-1. The secondary Ab consisted of FITC-conjugated anti-rat IgG F(ab')2 (Cappel, Durham, NC). Negative controls consisted of fluorescence obtained by staining cells with FITC-conjugated secondary Ab alone. The expression of CD3, CD4, CD44, and CD8 was detected by staining the cells with PE- or FITC-conjugated Ab against these markers for 30 min on ice, followed by washing three times. Nonspecific staining was blocked by incubation of cells with 0.5% normal mouse serum for 30 min before staining with labeled Ab. Next, 10,000 cells were analyzed by flow cytometry (EPICS V, model 752, Miami, FL).

Quantitation of VLS

Vascular leak was studied by measuring the extravasation of Evans blue dye, which when given i.v. binds to plasma proteins, particularly albumin, and following extravasation can be detected in various organs as described previously (14). Vascular leak was induced by injecting IL-2 as previously described (12). Groups of five mice were injected i.p. with 65,000 U of rIL-2 or PBS as a control, three times a day for 3 days. On day 4 they received one injection in the morning and 2 h later were injected i.v. with 0.1 ml of 1% Evans blue in PBS. After 2 h the mice were bled to death under anesthesia, and the heart was perfused with hepaticin in PBS as described previously (15). The lungs and liver, where maximum extravasation is known to occur, were harvested and placed in formamide at 37°C overnight. The Evan’s blue in the organs was quantitated by measuring the absorbance of the supernatant at 650 nm with a spectrophotometer. The VLS seen in IL-2-treated mice was expressed as the percent increase in extravasation compared with that in PBS-treated controls and was calculated as: ([µg of dye in the organ of IL-2-treated mice] – [µg of dye in the organ of PBS-treated control])/µg of dye in the organ of PBS-treated control × 100. Each mouse was individually analyzed for vascular leak, and the data from five mice were pooled and expressed as the mean ± SEM percent increase in VLS in IL-2-treated mice compared with that in PBS-treated controls.

Use of anti-CD44 mAbs to block VLS

C57BL/6 wild-type mice were treated with IL-2 as described above along with 100 µg F(ab')2 of anti-CD44 (IM7) Abs/mouse/day for a period of 4 days. The control mice received IL-2 and 100 µg of normal rat IgG/mouse/day for a similar duration. In a similar experiment mice were also injected with IL-2 plus 500 µg of F(ab')2 of anti-CD44 mAbs or IL-2 plus 500 µg of normal rat IgG as a control. The VLS seen in these IL-2-treated mice was compared with the VLS seen in PBS-treated mice, and the data were expressed as the percent increase in extravasation as described above.

Histology

For histopathological studies, groups of five separate mice were injected with IL-2 or PBS as described earlier, and on day 4 lungs and liver were fixed in 10% formalin solution. The organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Perivascular infiltration was scaled by counting the number of lymphocytes infiltrating the vessel and averaging the minimum and maximum range for each group (12). Three samples were used for lung, and 10 samples were used for the liver.

Electron microscopy studies

Tissue samples were fixed in 5% glutaldehyde/4.4% formaldehyde/2.75% picric acid in 0.05 M sodium cacodylate buffer, pH 7.4. Samples were washed in a sodium cacodylate buffer. Samples were postfixed in osmium tetroxide, embedded in Polybed 812 resin (Polysciences, Warrington, PA), and studied with an electron microscope (12).

Adoptive transfer of cells into CD44 KO mice

Spleen cells (2×10⁶ cells) from CD44⁻/⁻ (wild-type) or CD44 KO mice were injected i.p. into groups of four or five CD44-KO mice. These mice were injected with IL-2 as discussed above to induce VLS. After 4 days, the lungs and liver were harvested, and VLS was measured using the dye extravasation assay as described above. The IL-2-induced VLS was compared with that induced in PBS-treated CD44 KO mice, and the percent increase in extravasation was calculated as described above.

Proliferative responsiveness to HA

The proliferative responsiveness to HA was studied as described at length previously (16). Briefly, spleen cells from CD44⁻/⁻ (wild-type) or CD44 KO mice were cultured at a concentration of 6×10⁶ cells/well in 96-well flat-bottom plates in 200 µl of medium. The cells were cultured in various concentrations of HA (Sigma) for 48 h at 37°C. The cultures were pulsed with [3H]thymidine 6 h before harvesting using a cell harvester (Skatron, Sterling, VA), and the labeled DNA was counted in a liquid scintillation counter (16).

Mitogen stimulation

Spleen cells (1×10⁶) from CD44⁻/⁻ (wild-type) or CD44 KO mice were cultured in the presence of Con A (5 µg/ml), anti-CD44 mAbs (1/100 final dilution of hybridoma supernatant), or LPS (100 µg/ml) for 48 h in 0.2 ml of medium in multiple wells of tissue culture plates as previously described (16). The cultures were pulsed with [3H]thymidine 6 h before harvesting using the cell harvester, and the labeled DNA was counted in a liquid scintillation counter as described above.

Generation of LAK cells

Nylon wool-nonadherent spleen cells from CD44 KO and CD44 wild-type mice were cultured for 48 h with 1000 U/ml of IL-2. The cells were harvested, and viable cells were purified on Ficoll-Hypaque (Sigma) density gradient centrifugation. The cells were next tested for cytotoxicity against P815 tumor targets. In some experiments anti-CD44 mAbs (5 µg/ml) were added at the initiation of LAK cell cultures with IL-2. Also, in other experiments anti-CD44 mAbs (5 µg/ml) were added while testing the cytotoxicity of LAK cells against P815 tumor targets. In the above experiments normal rat IgG (5 µg/ml) was used as a control for anti-CD44 mAbs.

Cytotoxicity

The ability of splenic T cells to lyse various tumor targets was tested using the 51Cr release assay (17, 18). Briefly, 5×10⁶ target cells (P815) were labeled with 11NaCrO₄ by incubating at 37°C for 1 h. Varying E:T cell ratios in triplicate were added in 96-well round-bottom plates (Falcon 3910, Becton Dickinson, Lincoln Park, NJ) and incubated for 4 h at 37°C. Spontaneous release was measured by incubating the 51Cr-labeled targets alone, and total release was determined by incubating the labeled target cells with 0.1 M SDS. The supernatants were harvested after 4 h, and radioactivity was measured with a gamma counter (TriAnalytic, Elk Grove Village, IL).

Statistical analysis

The VLS data in different strains of mice were compared using ANOVA, and p < 0.05 was considered to be statistically significant.

Results

Detection of CD44 in wild-type and KO mice

To phenotypically confirm the expression of CD44, the wild-type and CD44 KO mice were screened using flow cytometry. To this end, splenic T cells from wild-type or CD44 KO mice were stained with mAbs against CD44 either immediately or following activation with Con A. The data shown in Fig. 1 indicated that T cells from CD44 KO mice failed to express CD44.
CD44 KO mice failed to express CD44 even after activation, while similar cells from wild-type mice expressed high levels of CD44, which was further up-regulated following activation with Con A.

**Decreased proliferative response of B cells to HA in CD44 KO mice**

Previous studies from our laboratory demonstrated that splenic B cells exhibit a strong proliferative response to stimulation with hyaluronate, an important ligand for CD44 (12). We therefore investigated whether the CD44 KO mice displayed a decreased proliferative response to HA. To this end, spleen cells were cultured in triplicate at a concentration of $6 \times 10^5$ cells/well. Medium alone (control) or HA was added to the wells, and the cultures were incubated at 37°C for 48 h. The cultures were pulsed with $[^{3}H]$thymidine (2 μCi) 6 h before harvesting. The cells were harvested using a semiautomated cell harvester, and the labeled DNA was counted in a liquid scintillation counter. The vertical bars represent the mean counts per minute ± SEM of triplicate cultures.

**Decreased VLS in CD44 KO mice**

To investigate the role of CD44 in IL-2-induced VLS, groups of five wild-type or CD44 KO mice were injected with 65,000 U of IL-2 three times a day for 3 days and once on day 4. On the last day, the mice were injected with 1% Evans blue dye, and VLS was studied by determining the extravasation of Evans blue in the lungs and liver.

Fig. 3 shows a representative experiment in which the wild-type (B6/6+/+) mice displayed significant VLS following IL-2 administration in the lungs and liver compared with the PBS-treated group. However, in the CD44 KO mice, there was a statistically significant decrease in VLS in the lungs and liver. These experiments were repeated three times with consistent results. These data suggested that CD44 may play a key role in the induction of VLS in the lung and liver.

**Histopathological studies in organs exhibiting VLS**

Inasmuch as CD44 is involved in lymphocyte homing to organs, histopathologic studies were conducted to investigate whether the decrease in VLS in CD44 KO mice was due to the inability of the lymphocytes to migrate to the lungs and liver. Mice were injected with PBS or IL-2 as described in Fig. 3. On day 4 the organs were harvested and stained with hematoxylin and eosin. The PBS-treated mice did not exhibit any perivascular infiltration in the lungs and liver (Fig. 4). In contrast, IL-2 treated wild-type mice

**FIGURE 1.** Detection of CD44 in the spleen using flow cytometry: CD44 wild-type (+/+; A1 and B1) and CD44 KO (−/−; A2 and B2) mice were screened for CD44 expression before (A) and after (B) stimulation with Con A via flow cytometry. Purified splenic T cells were stained for CD44 using PE-labeled anti-CD44 mAbs. The bold histograms represent unstained cells, and the broken histograms represent cells stained with PE-CD44.

**FIGURE 2.** Spleen cells from CD44 KO mice exhibit a decreased response to HA. Spleen cells from CD44 wild-type (+/+; 1) or CD44 KO (−/−; 2) mice were cultured in triplicate at a concentration of $6 \times 10^5$ cells/well. Medium alone (control) or HA was added to the wells, and the cultures were incubated at 37°C for 48 h. The cultures were pulsed with $[^{3}H]$thymidine (2 μCi) 6 h before harvesting. The cells were harvested using a semiautomated cell harvester, and the labeled DNA was counted in a liquid scintillation counter. The vertical bars represent the mean counts per minute ± SEM of triplicate cultures.

**FIGURE 3.** Decreased VLS in CD44 KO mice following IL-2 administration. Groups of five C57BL6 wild-type (+/+) and CD44 KO mice were injected i.p. with 65,000 U of IL-2 three times daily for 3 days and once on day 4. Two hours later the mice were injected with 1% Evans blue, and VLS was studied by determining the extravasation of Evans blue dye in the lungs and liver. The vertical bars represent the percent increase in VLS ± SEM seen following IL-2 treatment compared with that in the PBS-treated controls as described in Materials and Methods.
exhibited significant perivascular lymphocytic infiltration in the lungs (Fig. 4) and liver (Fig. 5), consistent with our earlier studies (12). It should be noted that the infiltration was seen only around venules, but not around arteries or arterioles. The IL-2-treated CD44 KO mice exhibited similar levels of perivascular infiltration as the IL-2-treated wild-type mice. The degree of infiltration was also measured by counting the number of lymphocytes infiltrating each vessel and averaging the range for each group (Table I). These results showed that IL-2-treated wild-type and CD44 KO mice had similar levels of perivascular infiltration. These data suggested that the decrease in IL-2-induced VLS seen in CD44 KO mice was not due to the inability of lymphocytes to migrate to the lungs and liver. IL-2 treatment also caused an increase in the density expression of LFA-1 in the lymphocytes (data not shown). It should be noted that the percentages of CD4+ and CD8+ T cells in wild-type and CD44 KO mice were similar before IL-2 treatment. Also after IL-2 treatment, there was a similar increase in the percentage of CD8+ T cells and a decrease in the percentage of CD4+ T cells in wild-type and CD44 KO mice (data not shown) as seen in our earlier study on wild-type mice (12).

Ultrastructural studies of the lung and liver from mice undergoing VLS

To further corroborate that IL-2-induced VLS resulted from actual damage to the endothelial cells, ultrastructural studies of the lung (Fig. 6) and liver (Fig. 7) were performed. As shown in Fig. 6 (panel 1), lungs from wild-type mice injected with PBS (control) displayed no morphological indications of damage to the endothelial cells. Similar results were seen in lungs of PBS-treated CD44 KO mice (Fig. 6, panel 3). In contrast, following injection with IL-2, the wild-type mice demonstrated extensive damage to the basal lamina and endothelial cells (Fig. 6, panel 2). Cellular debris from endothelial cells was found in the blood capillary lumen. Some of the endothelial cells were severely damaged, with only extended cell membrane remnants remaining (Fig. 6, panel 2). In contrast to the wild-type mice, IL-2-treated CD44 KO mice exhibited morphologically normal endothelial cells pressed against the basal lamina in the lungs (Fig. 6, panel 4). The endothelial cells had intact organelles, and very few morphological differences could be seen between the IL-2-treated (Fig. 6, panel 4) and control tissues from CD44 KO mice (Fig. 6, panel 3). These results corroborated the earlier observation that IL-2 treatment induces marked VLS in wild-type, but not CD44 KO, mice (Fig. 3). Also, the VLS data correlated with the actual damage to the endothelial cells.
Adoptive transfer of CD44\(^+\), but not CD44\(^-\), spleen cells along with IL-2 triggers VLS in CD44 KO mice

Although the decreased VLS seen in CD44 KO mice suggested an incriminatory role of CD44, it was not clear whether it could be attributed to the deficiency of CD44 on immune cells or nonimmune cells, including the endothelial cells. To address this, \(10^8\) spleen cells from CD44\(^+\) wild-type or CD44 KO mice were injected into CD44 KO mice, which also received IL-2, as described in Fig. 3. Next, VLS was measured in the lungs and liver. The data shown in Fig. 8 demonstrated that transfer of only CD44\(^+\), but not CD44\(^-\), spleen cells into IL-2-treated CD44-KO mice induced VLS in the lungs and liver. These data indicated that CD44 expression on immune cells was directly responsible for VLS induction.

Use of F(ab\(^9\))\(_2\) of Abs against CD44 on VLS induction in wild-type mice

To further confirm the role of CD44 in VLS, C57BL/6 wild-type mice were treated with IL-2 and 100 \(\mu\)g F(ab\(^9\))\(_2\) of Abs against CD44 (IM7) or IL-2 and with rat IgG as a control. As shown in Fig. 9A, injection of F(ab\(^9\))\(_2\) of Abs against CD44 significantly inhibited VLS in the lung and liver compared with the VLS in mice injected with IL-2 and control Abs. The inhibition caused by anti-CD44 in VLS was dose dependent as shown in Fig. 9. For example, in a similar experiment using 500 \(\mu\)g of F(ab\(^9\))\(_2\) of Abs against CD44 (Fig. 9B) we noted a greater inhibition of VLS in the lungs and liver compared with the controls. These data further support a role for CD44 in the induction of VLS.

Decreased LAK activity in CD44 KO mice

Previous studies from our laboratory and elsewhere have suggested that activated CTL and NK cells can use CD44 to kill the target cells (9, 10, 17, 18). We therefore investigated the LAK activity in CD44 KO mice. Splenic T cells were cultured with IL-2 and after 48 h tested for LAK activity against P815, an NK-resistant target. We noted that IL-2-activated LAK cells from CD44-KO mice exhibited decreased ability to kill P815 compared with the wild-type mice (Fig. 10A). We also tested whether CD44 expression on LAK cells was required for activation to the cytolytic phenotype or whether CD44 played an important role during

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Table I. Perivascular infiltration of lymphocytes in wild-type and CD44-KO mice treated with IL-2

<table>
<thead>
<tr>
<th>Organ</th>
<th>Strain(^a)</th>
<th>Wild type</th>
<th>CD44-KO</th>
</tr>
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<tbody>
<tr>
<td>Lung(^b)</td>
<td>3.85 ± 0.75</td>
<td>3.0 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>Liver(^c)</td>
<td>3.25 ± 0.27</td>
<td>2.81 ± 0.37</td>
<td></td>
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</tbody>
</table>

\(^a\) Mice were injected with IL-2 to induce VLS and the organs were processed for histopathological studies as described in Materials and Methods. The degree of perivascular infiltration was measured by counting the number of lymphocytes infiltrating each vessel.

\(^b,c\) The number of lymphocytes infiltrating a venule. The data represent the mean ± SEM obtained from four samples per mouse (four mice were analyzed for each organ).
the effector phase in mediating the cytotoxicity. To this end, spleen cells from CD44 wild-type mice were cultured with IL-2 to generate LAK cells in the presence of Abs against CD44. In addition, some spleen cells from CD44 wild-type mice were cultured with IL-2 alone to generate LAK cells. Subsequently, when testing the cytolytic activity of such LAK cells against P815 target cells, they were cultured in the presence of anti-CD44 mAbs. In the above studies normal rat IgG was used as a control for anti-CD44 mAbs. The data shown in Fig. 10 indicated that the presence of anti-CD44 mAbs during the generation of LAK cells did not influence the level of LAK activity. In contrast, addition of anti-CD44 mAbs during the cytotoxicity testing blocked significantly LAK activity (Fig. 10C). These data together demonstrated that CD44 expression played a critical role at the effector phase of LAK cell function rather than at the differentiation phase.

**Discussion**

In the current study we observed that IL-2-induced VLS in the lungs and liver was markedly decreased in CD44 KO mice.
compared with that in wild-type mice. Furthermore, ultrastructural studies demonstrated that unlike the IL-2-injected wild-type mice, CD44 KO mice failed to exhibit endothelial cell damage. These data together demonstrated that CD44 is actively involved in the induction of VLS by IL-2. The fact that adoptive transfer of CD44\(^1\), but not CD44\(^2\), spleen cells along with IL-2 into CD44 KO mice induced VLS as well as the observation that administration of F(ab')\(_2\) of anti-CD44 mAbs could block the VLS further corroborated the role played by CD44 in VLS induction. These data also suggested that blocking CD44 may serve as a useful therapeutic approach to prevent endothelial cell injury in a variety of clinical conditions.

There are many disease models in which factors other than cytolytic lymphocytes have been shown to participate in endothelial cell injury, including neutrophils and complement components (19). However, there is growing evidence for the involvement of cytolytic lymphocytes in endothelial cell injury. For example, IL-2-activated T cells and other leukocytes have been shown kill endothelial cells (20–24). Also, IL-2-induced VLS was seen only in immunocompetent, not in nude or immunodeficient, mice (25, 26). Furthermore, depletion of NK/LAK cells in vivo led to decreased toxicity during IL-2 therapy (27). Studies from our laboratory demonstrated that administration of a CTL clone plus IL-2 into irradiated syngeneic mice, but not the CTL clone or IL-2 alone.
triggered VLS (21). Moreover, we have demonstrated that perforin-deficient or Fas ligand-defective mice exhibit decreased VLS in the lungs and liver following IL-2 therapy (12). Although all the studies mentioned above indicate the role of cytotoxic lymphocytes in endothelial cell injury and consequent induction of VLS, the exact mechanism of endothelial cell damage is not clear.

We and others have shown that activated CTL, NK cells, and double-negative T cells found in mice with the lpr mutation mediate efficient lysis of target cells when activated through CD44 (10, 11, 18). Inasmuch as, endothelial cells express the ligands for CD44, it is likely that dysregulated interaction between cytotoxic lymphocytes and endothelial cells can cause endothelial cell lysis. The current study supports this hypothesis. IL-2 treatment activates the LAK cells to express higher levels of perforin and Fas ligand as well as CD44, which may account for their ability to migrate to various organs and cause endothelial cell lysis (12, 28).

It is likely that CTL/LAK-induced endothelial cell damage may occur at sites of chronic inflammation or following cytokine therapy, but not during normal immune responses. This can be explained by regulatory mechanisms operating in vivo during the normal immune response. First, CD44 expression is up- or downregulated based on the stage of activation of lymphocytes.
Secondly, CD44 is expressed in a variety of isoforms, and therefore, the isoforms involved in cytotoxicity, homing, and adhesion may be different. Thirdly, CD44 and its ligand, HA, were found in soluble form in the serum and have been known to vary during disease conditions (10, 29). Such molecules may regulate the interactions between CTL and endothelial cells. Lastly, the interaction between Fas ligand and Fas expressed on cytotoxic lymphocytes and endothelial cells may regulate the degree of nonspecific cytotoxicity in vivo.

Although CD44 is known to play a wide variety of roles, mice deficient in CD44 do not exhibit developmental or neurological defects (13). However, such mice exhibit decreased LAK cell activity (30). This observation was confirmed in the current study, in which we noted that IL-2-activated LAK cells from CD44 mice, compared with those from wild-type mice, exhibited a marked decrease in their ability to kill NK-resistant P815 target cells. In the current study we noted that the IL-2-treated CD44 KO mice exhibited similar levels of perivascular infiltration with lymphocytes in the lungs and liver compared with wild-type mice. These data together suggested that the decreased VLS in CD44 KO mice resulted from the inability of LAK cells to mediate lysis of endothelial cells rather than the inability of lymphocytes to migrate to and home to various organs. This was further corroborated by ultrastructural studies in which endothelial cells from CD44 KO mice showed normal morphology, unlike similar cells from wild-type mice, which exhibited marked damage. Recent studies have suggested that expressed by LAK cells may recognize HA on target cells to mediate cytotoxicity (30). In an earlier study we noted that B lymphocytes when activated with HA underwent proliferation and differentiation (16). In the current study we used CD44 KO mice to test whether such cells would exhibit decreased response to HA. The data demonstrated that the B cell responsiveness to HA in CD44 KO mice was significantly diminished, but not completely abrogated. These data further confirmed the role of CD44-hyaluronate interaction in lymphocyte activation. Also, because the responsiveness of B cells to HA in CD44 KO mice was not completely abrogated, alternate receptors, such as CD38, may play a role in B cell activation (31).

In the current study although IL-2-induced VLS was used as a model to study endothelial cell damage, there are a number of clinical diseases in which similar endothelial cell injury has been reported. In murine lymphocytic choriomeningitis viral infection, a massive delayed-type hypersensitivity reaction has been known to occur in the nervous system, caused by CD8$^+$ T cells (1). It has been speculated that virally activated CD8$^+$ T cells expressing high levels of CD44, kill endothelial cells, leading to massive extravasation of monocytes and CD44$^+$ T cells in the subarachnoid space. In autoimmune disease models involving vasculitides, the lesions are associated with infiltration of lymphocytes and macrophages at the vascular wall structure (3). The T cell involvement in the endothelial cell damage also leads to vascular disease in scleroderma (32). Moreover, the PBLs from some patients with rheumatoid arthritis and giant cell arteritis have been shown to be cytotoxic to endothelial cells, but not to fibroblasts (33). Further studies in such disease models on the role of CD44 should help in understanding and preventing pathogenesis.

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