Inflammation, Vol. 22, No. 4, 1998

CYTOKINE AND ADHESION MOLECULE REQUIREMENTS FOR LUNG INJURY INDUCED BY ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODY

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Abstract—Acute hemorrhagic lung injury occurs in humans with anti-GBM antibody (Goodpasture's syndrome), however, the mechanism of this injury is still largely unknown. To date, treatment has been confined to steroids and plasmaphoresis. Infusion of anti-GBM antibody into rats caused lung injury with intra-alveolar hemorrhage and intrapulmonary accumulation of neutrophils. Lung injury was dependent on the presence of neutrophils and complement and required both TNFα and IL-1. Experiments employing blocking antibodies to adhesion molecules demonstrated requirements for the β_1 integrin VLA-4, β_2 integrins LFA-1 and Mac-1, and L-selectin. The endothelial cell adhesion molecules, E-selectin and ICAM-1, were also required for the full development of lung injury. Inhibition of TNFα or IL-1 or adhesion molecules reduced both lung injury and tissue neutrophil accumulation. Thus, this study underscores cytokine and adhesion molecule requirements for neutrophil mediated injury in lung and kidney caused by anti-GBM, suggesting potential targets for the treatment of Goodpasture's syndrome in humans.

INTRODUCTION

The presence of circulating antibody to glomerular basement membrane (anti-GBM) in humans is known to be associated with the development of both renal glomerular and pulmonary vascular adnormalities (1–6). The clinical pattern of anti-GBM-related nephritis varies from a relatively mild focal glomerulonephritis with hematuria to crescentic glomerulonephritis rapidly progressing to renal failure (7). In these patients there may also be pulmonary manifestations

0360-3997/98/0800-0403$15.00/0

that are associated with acute, life-threatening bouts of intra-alveolar hemorrhage and diffuse alveolar damage (Goodpasture's syndrome). It is known experimentally that sheep or rabbit antibody to rat glomerular GBM reacts with both renal and lung vascular basement membranes (2, 7, 8). The chief basement membrane component that is reactive with anti-GBM antibody appears to be the $\alpha 3$ chain of type IV collagen (9). Polyclonal IgG sheep antibodies to rat GBM, to murine type IV collagen, and to laminin produce evidence of acute lung injury, coincident with fixation of the antibody to the pulmonary vasculature (10, 11).

In the current studies we employed a rat model to define the mechanisms of acute lung injury following infusion of sheep anti-rat-GBM. The data demonstrate requirements of cytokines and adhesion molecules in the development of anti-GBM-induced lung injury. These studies delineate many similarities but also some distinct differences with respect to mediators required for induction of acute lung injury and development of acute renal glomerular injury. These findings may provide insight into the development of new therapeutic strategies for the treatment of humans with Goodpasture's syndrome.

METHODS

Animal Model of Anti-GBM-Induced Acute Alveolitis. Sheep polyclonal IgG rich in anti-rat GBM was prepared by immunization of sheep with rat GBM as previously described (12). Male Long-Evans specific pathogen-free rats (300–350 g; Charles River Breeding Laboratories, Portage, Michigan) were used for all studies. Intraperitoneal ketamine (100 mg/kg) was used for sedation and anesthesia. For studies of lung vascular permeability, 10 mg sheep IgG anti-GBM was mixed with 0.5 μCi ^{125}I-BSA and injected intravenously in a total volume of 0.5 ml. Negative control rats received 10 mg of sheep IgG obtained from pre-immune serum. For studies of alveolar hemorrhage, 0.5 μCi of ^{51}Cr-rat RBC was injected intravenously prior to injection of anti-GBM. The anti-GBM preparations contained very low levels (650 fg) of endotoxin activity, as measured by the Limulus amebocyte lysate assay (Sigma Chemical Co., St. Louis, Missouri). Rats were sacrificed 6 h after injection of anti-GBM and the pulmonary circulation was flushed with 10 ml saline by pulmonary artery injection. Changes in lung vascular permeability or alveolar hemorrhage were assessed by comparing ^{125}I- or ^{51}Cr-radioactivity in lungs to counts present in 1.0 ml blood. Positive controls received either anti-GBM alone for complement and neutrophil depletion studies, 200 μg non-specific MOPC-21 IgG_1 intravenously for protocols employing blocking monoclonal antibodies to leukocyte and endothelial cell adhesion molecules, or 1.0 ml preimmune serum intravenously for cytokine requirement studies.

Strategies for in vivo Blockade of Adhesion Molecules and Cytokines. The dosing schedules for blocking of the rat β_1 integrin CD49d; rat β_2 integrins, CD11a, CD11b, and CD18; rat E- and L-selectins; rat ICAM-1, and rat TNFα and IL-1 were determined on the basis of previous studies in which these blocking agents were administered intravenously and shown to block inflammatory reactions after intrapulmonary deposition of IgG immune complexes. The blocking agents are described below.

MAb to Rat VLA-4 (CD49d). Anti-rat CD49d, clone TA-2, was a gift from Dr. Thomas Issekutz (Dalhousie University, Halifax, Canada). This antibody is of the IgG_1 subclass and is reative

with the $\alpha 4$ chain (CD49d) of β_1 integrin. It blocks binding of rat lymphocytes to cytokine-activated vascular endothelial cells (13). When used, 800 μg (0.25 ml) TA-2 IgG_1 were infused intravenously just prior to, and 4 h after infusion of anti-GBM. Using this protocol, a single intravenous injection of this antibody strongly inhibits lymphocytes accumulation in delayed-type cutaneous hypersensitivity reactions in rats (14).

MAb to Rat LFA-1 (CD11a). Anti-rat CD11a, clone WT-1, is of the $IgG_{2\alpha}$ sublcass and recognizes a leukocyte epitope of 160 to 170 kDa, but does not react with the 95 kDa β-subunit of LFA-1 (15). WT-1 blocks binding of rat lymphocytes to cultured endothelial cells obtained from venules in rat lymph nodes as described elsewhere (16). When used, 200 μg intact WT-1 were injected intravenously immediately prior to infusion of anti-GBM.

MAb to Rat Mac-1 (CD11b). Anti-rat CD11b, clone 1B6c, is of the murine IgG_1 subclass, and has been previously characterized (17). It does not bind to rat lymphocytes but blocks aggregation of PMA-activated rat neutrophils. A total of 200 μg intact 1B6c was injected intravenously immediately prior to infusion of anti-GBM.

MAb to Rat CD18. Anti-rat CD18, clone CL26, was a gift from Dr. C. Wayne Smith (Baylor College of Medicine, Houston, Texas). This antibody is of the murine IgG_1 subclass and binds to rat neutrophils, lymphocytes, and monocytes. It reacts with a 95-kDa protein, consistent with the β-chain of CD18 (18). Since intravenous infusion of intact IgG_1 CL-26 produced neutropenia (17), $F(ab')_2$ fragments were prepared and were found not to induce neutropenia. When used, 67 μg $F(ab')_2$ anti-CD18 were injected intravenously 2, 4 and 5 h after infusion of anti-GBM.

MAb to Rat E-selectin. Anti-E-selectin, clone CL-3, was a gift from Dr. Donald C. Anderson (Upjohn Co., Kalamazoo, Michigan). The $F(ab')_2$ preparation rather than intact IgG_1 was used, since the intact antibody neither prevents adherence of phorbol ester-stimulated rat blood neutrophils to TNFα-treated RPAEC nor does it protect against IgG immune complex-induced acute lung injury (19). When used, 67 μg $F(ab')_2$ CL-3 were injected intravenously 2, 4 and 5 h after infusion of anti-GBM.

MAb Rat L-selectin (HRL1). Hamster mAb to rat L-selectin, clone HRL1, was generated as previously described (20). This antibody is of the IgG_1 subclass and reacts with EL-4 cells transfected with the cDNA for rat L-selectin but not with El-4 cells transfected with vector alone. HRL1 also blocks binding of yeast polymannon [containing the polyphosphomannon ester (PPME)] to rat lymphocytes and blocks binding of rat endothelial cells (derived from high endothelial venules of rat lymph nodes) to solid phase rat L-selectin-Ig affixed to the surfaces of plastic dishes. Because intact anti-L-selectin caused neutropenia in rats, $F(ab')_2$ fragments were employed as described previously (20). When used, 67 μg $F(ab')_2$ HRL1 were injected intravenously 2, 4 and 5 h after infusion of anti-GBM.

MAb to Rat ICAM-1. Anti-rat ICAM-1, clone 1A29, recognizes rat ICAM-1 based on: 1) its ability to inhibit homotypic aggregation of T-cell blasts induced by addition of phytohemagglutinin; 2) antigen precipitation analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; 3) by antigen distribution on frozen sections of postcapillary venules; and 4) cytokine-induced upregulation of this antigen on rat endothelial cells (21). When used in vivo for blocking activity, the antibody was used as $F(ab')_2$ fragments since the intact antibody does not interfere with adhesion interactions between neutrophils and the activated endothelium. When used, 67 μg $F(ab')_2$ IA29 were injected intravenously 2, 4 and 5 h after infusion of anti-GBM.

Polyclonal Anti-TNFα. Anti-TNFα polyclonal goat IgG antibody was raised to recombinant murine TNFα and found to cross-react with rat TNFα (22). When used, 1.0 ml immune goat serum was injected intravenously immediately after infusion of anti-GBM. Anti-TNFα treatment blocks appearance of TNFα in rat bronchoalveolar fluids after intrapulmonary deposition of IgG immune complexes (22).

Interleukin-1 Receptor Antagonist. The preparation of human recombinant IL-1 receptor antagonist (IL-1Ra) has been previously described (23). Subcutaneous injection of IL-1Ra (2 mg/kg)

just prior to infusion of anti-GBM, followed by intravenous injections of 1 mg/kg 2, 4, and 5 h after infusion of anti-GBM was in accord with an established protocol that provides profound IL-1 receptor blockade in rats (12).

Lipopolysaccharide (LPS) Content of Monoclonal Antibodies. All MAb were evaluated for LPS content using a Limulus amebocyte lysate assay (Sigma Chemical Co.). On the basis of the amount used for each antibody (given intravenously), the amounts of endotoxin equivalent used in vivo were: 6 ng for anti-VLA-4 (TA-2), 10 ng for anti-CD11a (WT1), 6.8 ng for anti-CD11b (1B6c), 1 ng for anti-CD18, 1.4 ng for anti-L-selectin (HRL-1), 1 pg for anti-GBM, 1.2 ng for anti-E-selectin and 96 pg for anti-ICAM-1 (1A29). The amounts for MOPC-21, pre-immune control serum and anti-TNFα were all less than 10 pg.

Complement Blockade and Neutrophil Depletion. Complement blockade was achieved by the use of human recombinant sCR1 (kindly provided by Dr. Una Ryan, T-Cell Sciences, Cambridge, Massachusetts). Human recombinant sCR1 was injected subcutaneously (15 mg/kg) into rats 6 h before anti-GBM. This dose has been shown to protect against complement-mediated tissue injury (23). Neutrophil depletion was achieved by intraperitoneal injection of 1.0 ml rabbit anti-rat neutrophil antibody (Accurate Chemical and Scientific Corp., Westburg, New York) 16 h before infusion of anti-GBM. This technique reduces blood neutrophil counts by >99% (to <10 neutrophil/mm^3 blood).

Tissue Extraction of MPO Content. Tissue samples were extracted by homogenization and sonication in phosphate buffer. MPO activity in supernatants was measured by the change in optical density (at 460 nm) resulting from decomposition of H_2O_2 in the presence of *O*-dianisidine (22).

Immunofluorescence Analysis of Lung. Lungs were inflated with optimal cutting temperature (OCT) compound. Frozen sections were acetone-fixed, washed in PBS and then incubated with FITC-rabbit anti-sheep IgG. After incubation for 10 min at 27°C, sections were washed in PBS, coverslips attached and examined by fluorescence microscopy.

Lung Morphology. Six hours after infusion of anti-GBM, lungs were inflated with 10% buffered formalin and embedded in plastic. Tissue sections (1 μm) were obtained and stained with toluidine blue for morphologic evaluation.

Statistical Analysis. All values are expressed as mean ± *SEM*. Data were analyzed with a one-way analysis (ANOVA) of variance and individual group means were then compared with a Tukey's test. Differences were considered significant when $P < 0.05$. For calculations of percent change, negative control values were subtracted from positive control and treatment group values.

RESULTS

Lung Injury Induced by Sheep IgG Anti-GBM. Intravenous infusion of 10 mg IgG anti-GBM resulted in acute lung injury as assessed by lung permeability, alveolar hemorrhage and lung MPO content (Figure 1). In all cases, the differences between the two groups were statistically significant ($P < 0.05$), with values from anti-GBM-treated rats being 3 to 7 fold greater than the values from rats treated with normal sheep IgG. When frozen sections of lung from each of the two groups (anti-GBM versus normal sheep IgG) were stained for presence of sheep IgG, no fixation was found in rats infused with normal sheep IgG (Figure 2A). However, the expected pattern of linear vascular deposition of sheep IgG was observed in lung from rats infused with anti-GBM (Figure 2B).

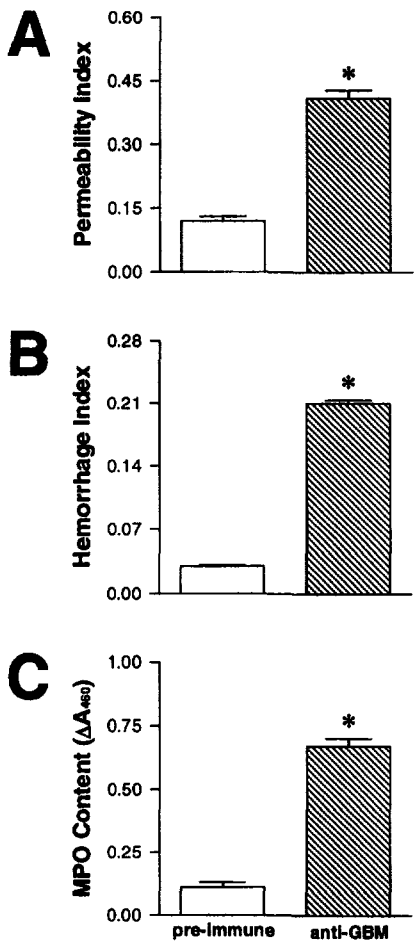

Fig. 1. Effects of anti-GBM infusion on lung vascular permeability (A), alveolar hemorrhage (B), and neutrophil accumulation (C). Negative controls received 10 mg sheep IgG from preimmune serum. Positive controls received 10 mg sheep IgG anti-GBM. For each group, $N = 4$. $^*P < 0.01$ compared to negative control group.

The morphological features of the reactions produced by intravenous infusion of anti-GBM in the lung are shown in Figure 3. Anti-GBM caused diffuse intra-alveolar hemorrhage along with accumulation of neutrophils, which were largely present within venules and interstitial capillaries, although some were found in intra-alveolar locations. The infusion of 10 mg sheep IgG anti-rat GBM has also been shown to induce nephrotoxic nephritis with marked proteinuria within 24 h (12).

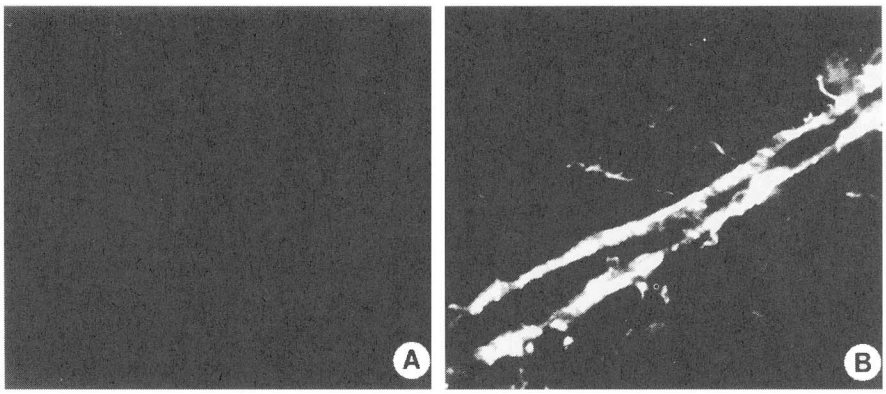

Fig. 2. Fluorescence immunostaining of rat lungs for sheep IgG 4 h after infusion of 10 mg IgG normal sheep IgG (A) or sheep IgG anti-GBM (B). No staining was found with after infusion of normal sheep IgG whereas, in animals infused with anti-GBM, diffuse vascular (venular) staining for sheep IgG was present (× 120).

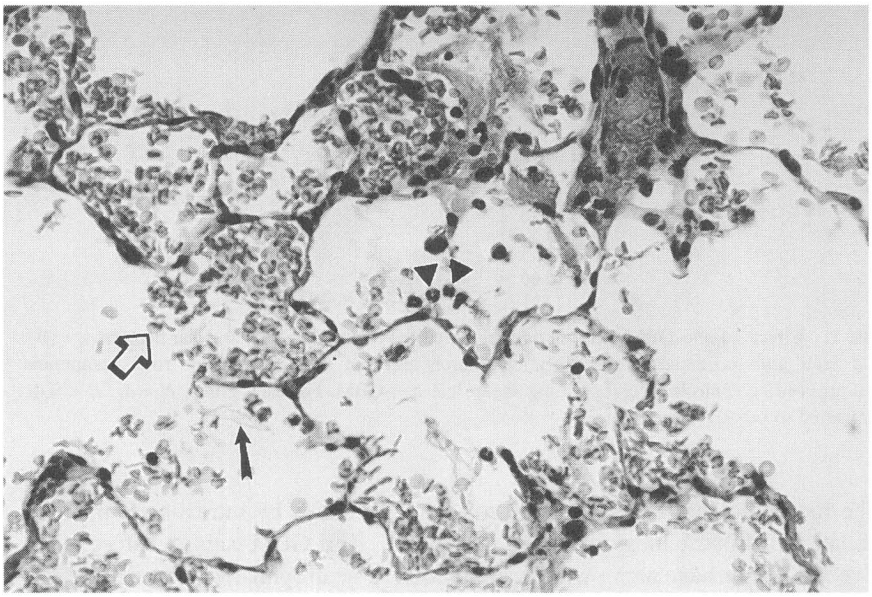

Fig. 3. Morphological features of lung from rats infused intravenously with 10 mg anti-GBM. Extensive intra-alveolar hemorrhage (open arrow) and fibrin deposition (closed arrow) was consistently observed. Neutrophils were present within interstitial capillaries, as well as some within alveoli (arrowheads). (Toluidine blue, × 140.)

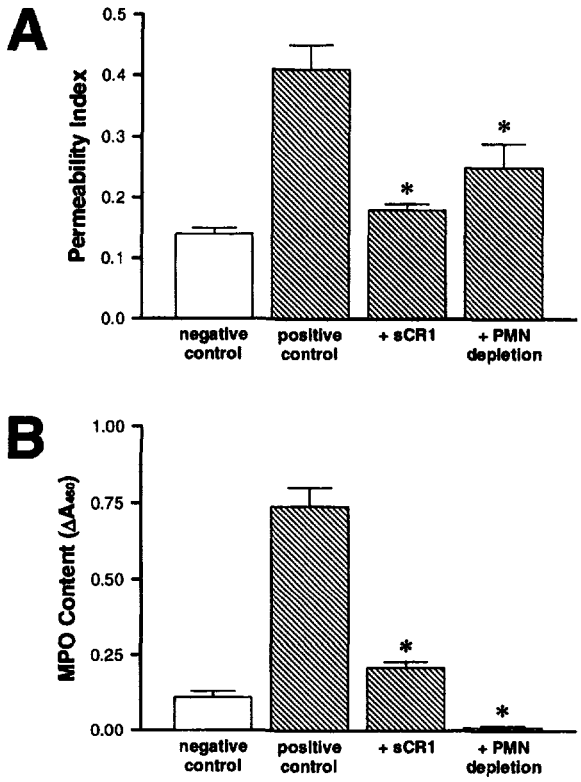

Fig. 4. Effects of complement blockade (sCR1) and neutrophil (PMN) depletion on anti-GBM-induced increases in lung vascular permeability (A) and neutrophil accumulation (B). For each group, $N = 4$–6. $^*P < 0.01$ compared to positive control group.

Requirements for Neutrophils and Complement for Lung Injury. Lung injury induced by anti-GBM infusion was found to be dependent upon complement, since treatment of rats with the complement-blocking reagent, sCR1, resulted in an 85% decrease in lung vascular permeability (Figure 4A). Similarly, depletion of blood neutrophils with antibody reduced anti-GBM-induced lung permeability by 62%. Consistent with reductions in lung permeability, both complement blockade (sCR1) and neutrophil depletion dramatically reduced anti-GBM-induced lung neutrophil accumulation as measured by MPO content (Figure 4B). Treatment with sCR1 reduced lung MPO values by 79%, while neutrophil depletion decreased MPO values by >95%, to nearly undetectable levels. Thus, it appears that both complement and neutrophils are required for lung injury induced by anti-GBM.

Cytokine Requirements for Lung Injury. Other models of lung injury have

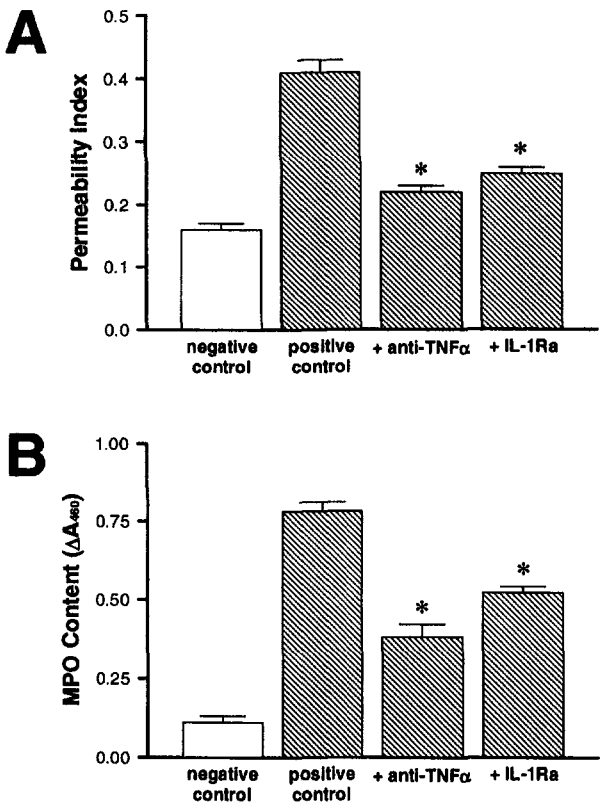

Fig. 5. Effects of TNFα and IL-1 blockade on anti-GBM-induced increases in lung vascular permeability (A) and neutrophil accumulation (B). For each group, $N = 4$. $^*P < 0.01$ compared to positive control group.

shown that the early response cytokines, TNFα and IL-1, are central to the lung inflammatory response (22, 24). To determine whether these cytokines participate in lung injury induced by anti-GBM antibody, rats were treated with anti-TNFα antibody or IL-1Ra. Increases in lung vascular permeability induced by anti-GBM were reduced by anti-TNFα and IL-1Ra by 76% and 64%, respectively (Figure 5A). Anti-TNFα and IL-1Ra had similar effects on anti-GBM-induced neutrophil recruitment into lung as measured by tissue MPO content; anti-TNFα and IL-1Ra reduced lung MPO values by 60% and 39%, respectively (Figure 5B). These data suggest that full development of lung injury after infusion of anti-GBM is dependent upon both TNFα and IL-1.

Requirements for Leukocyte Adhesion Molecules for Lung Injury. Experiments employing blocking antibodies to leukocyte adhesion molecules demon-

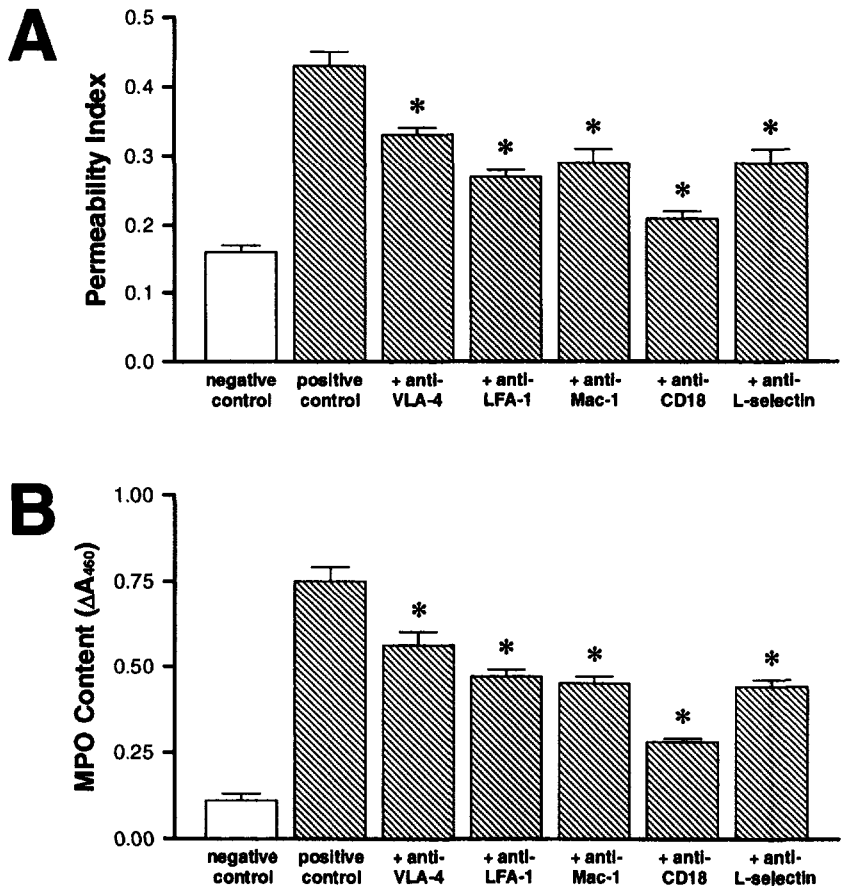

Fig. 6. Effects of leukocyte adhesion molecule blockade on anti-GBM-induced increases in lung vascular permeability (A) and neutrophil accumulation (B). For each group, $N = 4$–6. $^*P < 0.01$ compared to positive control group.

strated requirements for β_1 and β_2 integrins and L-selectin for the full induction of lung vascular permeability (Figure 6A). Treatment with antibody to the β_1 integrin VLA-4 (CD49d) resulted in a 37% reduction in anti-GBM-induced lung permeability. Similarly, antibodies to the β_2 integrins LFA-1 (CD11a), Mac-1 (CD11b) and the β_2 common chain (CD18) decreased permeability values by 59%, 51%, and 78%, respectively. Antibody to rat L-selectin reduced anti-GBM-induced lung permeability by 51%. Correlating with lung permeability studies, blockade of leukocyte adhesion molecules decreased neutrophil recruitment into lung (Figure 6B). Blockade of the β_1 integrin VLA-4 caused a 30%

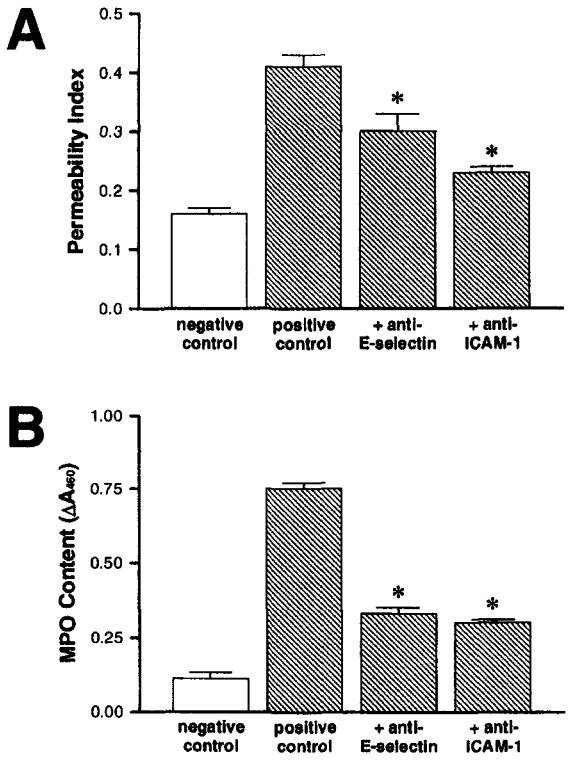

Fig. 7. Effects of endothelial cell adhesion molecule blockade on anti-GBM-induced increases in lung vascular permeability (A) and neutrophil accumulation (B). For each group, $N = 4$. $^*P < 0.01$ compared to positive control group.

decrease in lung MPO; blockade of the β_2 integrins LFA-1, Mac-1 and the β_2 common chain (CD18) reduced lung MPO by 44%, 47%, and 73%, respectively. Antibody to rat L-selectin produced a 48% reduction in lung MPO content. Thus, VLA-4, LFA-1, Mac-1 and L-selectin all appear to be required for the full expression of lung injury after infusion of anti-GBM.

Requirements for Endothelial Adhesion Molecules for Lung Injury. In other models of lung inflammatory injury, expression of E-selectin and ICAM-1 on the pulmonary endothelium is essential for full induction of tissue injury, this expression being induced by TNFα and IL-1 (19, 25). To assess the roles of E-selectin and ICAM-1 during anti-GBM-induced lung injury, rats were treated with blocking antibodies to these adhesion molecules. Antibodies to E-selectin and ICAM-1 were both effective in attenuating anti-GBM-induced vascular permeability, reducing index values by 39% and 74%, respectively (Figure 7A). Consistent with reduc-

tions in lung permeability, anti-E-selectin and anti-ICAM-1 decreased lung MPO content by 66% and 70%, respectively (Figure 7B). These data suggest that the full extent of lung injury after infusion of anti-GBM is dependent upon endothelial expression of E-selectin and ICAM-1.

DISCUSSION

Infusion of anti-GBM into rats caused acute lung injury, as defined by morphological analysis and by measurement of increased vascular permeability and tissue accumulation of neutrophils. Furthermore, fixation of anti-GBM to the pulmonary vascular basement membrane results in neutrophil- and complement-dependent injury that requires β_1 and β_2 integrins and ICAM-1. Additional requirements for L- and E-selectin in this model of injury are similar to lung injury induced by IgG immune complexes in which E- and L-selectins are involved in the recruitment of neutrophils (19, 20). Requirements for TNFα and IL-1 may reflect the fact that fixation of anti-GBM to the lung vasculature results in activation of residential interstitial and/or alveolar macrophages. This activation process could be the result of vascular formation of IgG immune complexes and the presence of complement activation products, causing macrophage release (perhaps in a VLA-4-dependent manner) of both TNFα and IL-1. In turn, these cytokines would cause upregulation of endothelial E-selectin and ICAM-1, thereby promoting adhesive interactions between neutrophils and the pulmonary vascular endothelium. Following recruitment of neutrophils along chemotactic gradients, vascular injury would presumably then be caused by neutrophil-derived toxic oxygen products and proteinases.

The cytokine and adhesion molecule requirements for anti-GBM-induced lung injury are similar in many respects to the requirements in anti-GBM-induced acute glomerular injury, as defined by proteinuria as well as by morphological changes (12). In the current studies of lung injury, the same amount of anti-GBM antibody was administered as was given in our previous studies of anti-GBM-induced glomerular injury (12) in order to facilitate comparison of cytokine and adhesion molecule requirements between lung and kidney. In both organs, anti-GBM-induced injury is neutrophil and complement-dependent (8). However, requirements for leukocyte adhesion molecules differ significantly in these two in vivo models. While Mac-1 is required for the full development of anti-GBM-induced acute glomerular injury, there was no requirement for LFA-1 in this form of glomerular injury, nor was IL-1 required for development of glomerular injury (12), which is in contrast to requirements for both Mac-1 and LFA-1 as well as TNFα and IL-1 in anti-GBM-induced lung injury (Figures 5 and 6). Despite this difference in β_2 integrin participation, both VLA-4

and L-selectin were required for full development of injury in both lung (Figure 6) and kidney (12).

Additional differences were evident in the requirements for endothelial cell adhesion molecules. As in the kidney (12), neutrophil accumulation and tissue injury in lung following infusion of anti-GBM requires ICAM-1 (Figure 7). However, E-selectin is not required in the kidney (12), while it is critical to the development of lung injury (Figure 7). These differences are supported by immunohistochemical analysis of injured renal tissues. In the kidney, anti-GBM caused very limited upregulation of glomerular vascular E-selectin. E-selectin did not play a measurable role in either the accumulation of neutrophils in glomerular capillaries or in anti-GBM-induced proteinuria. Conversely, ICAM-1 was strongly expressed (as defined by immunostaining) in renal glomeruli following infusion of anti-GBM (12).

In the context of cytokine dependency, there is a distinct difference in cytokine requirements for anti-GBM-induced lung versus glomerular injury. In both organs, there is an absolute requirement for TNFα. However, whereas blocking of IL-1 by IL-1Ra was protective in anti-GBM-induced lung injury (Figure 5), neither IL-1Ra nor anti-IL-1 was protective in the glomerular injury model (12). It has also been shown that renal arterial perfusion with TNFα induces strong expression of ICAM-1 and VCAM-1 in rat glomeruli, whereas infusion of IL-1 does neither (11). This suggests that the rat glomerulus may be relatively refractory to the effects of IL-1, at least under the conditions employed. It has also been suggested that IL-1Ra may reduce proteinuria and neutrophil accumulation in glomeruli after infusion of anti-GBM (26), however the amount of IL-1Ra used in those studies was 6-fold in excess of the amount used in our earlier studies (12). Whether anti-GBM preferentially causes glomerular expression of TNFα (but not IL-1) is not currently known.

When IgG immune complex-induced dermal vasculitis was induced simultaneously with similar induction of alveolitis, distinct differences in cytokine requirements were also found to be organ specific. In lung, both TNFα and IL-1 were required for neutrophil recruitment and full development of lung injury, whereas in the dermis IL-1, but not TNFα, was required (27). Immunostaining of the skin revealed the appearance of IL-1 in mast cells in the absence of TNFα. Thus while lung macrophages generate both TNFα and IL-1, dermal mast cells only express IL-1. Differences in organ production of early response cytokines implies that in human inflammatory conditions in which cytokines are thought to play a role, such subtle but significant differences may be important in the design of therapeutic interventions.

Anti-GBM-induced lung injury shares many similarities to requirements following intrapulmonary deposition of IgG immune complexes (BSA, anti-BSA). In both models, complement and neutrophils are required (28). However, only LFA-1 is required for lung neutrophil recruitment induced by IgG immune

complexes, while Mac-1 engagement involves activation of lung macrophages, as determined by effects of intravascular or airway instillation of antibodies to LFA-1 or Mac-1 (29). Anti-GBM-induced lung injury requires the participation of both LFA-1 and Mac-1 for neutrophil recruitment and full development of injury, as determined by intravenous administration of anti-LFA-1 or anti-Mac-1 (Figure 6). In a sense, the anti-GBM model of lung injury, which appears to be associated largely with intravascular (but little extravascular) accumulation of neutrophils, is similar to the model of lung injury following intravascular activation of complement, in which both LFA-1 and Mac-1 are required (27). The predominant intravascular activation and accumulation of neutrophils in the anti-GBM model may explain the reason for successful therapy with plasmaphoresis in patients with Goodpasture's syndrome.

The data presented in this report suggest that the cytokine and adhesion molecule requirements for anti-GBM-induced lung injury are similar to those found in other lung injury models. Furthermore, we have identified significant differences in cytokine and adhesion molecule requirements between anti-GBM induced lung and glomerular injury. While the reasons for these organ-specific responses are unclear, these findings may suggest fundamental differences in pathophysiological mechanisms involved in the development of pulmonary/renal syndromes and may help to define potential therapeutic interventions.

Acknowledgments—The authors would like to thank Mrs. Beverly Schumann for assistance in the preparation of this manuscript. We thank Drs. C. Wayne Smith (Baylor College of Medicine) and D. C. Anderson (Upjohn Co., Kalamazoo, Michigan) for the gifts of anti-CD18 and anti-E-selectin, Dr. T. Issekutz (Dalhousie University, Halifax, Canada) for the gift of anti-CD49d, and Dr. U. S. Ryan (T-Cell Sciences, Needham, Massachusetts) for the gift of sCR1. This study was supported by National Institutes of Health grants GM-29587 and HL-31963.

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