Inflamm. res. 47 (1998) 251–255
1023-3830/98/060251-05 $ 1.50+0.20/0

Inflammation Research

Cytokine and adhesion molecule requirements for neutrophil recruitment during glycogen-induced peritonitis

M. S. Mulligan[1], A. B. Lentsch[1], M. Miyasaka[2] and P. A. Ward[1]

[1]Department of Pathology, The University of Michigan Medical School, 1301 Catherine Road, Ann Arbor, MI 48109-0602, USA, Fax +1 313 763 4782, e-mail: pward@umich.edu
[2]Department of Bioregulation, Osaka University Medical School, Biomedical Research Center, 2-2 Yamadaoka, Suita 565, Japan

Received 8 December 1997; returned for revision 25 February 1998; accepted by G. W. Carter 6 March 1998

Abstract. *Objective:* Requirements for cytokines and adhesion molecules for perititoneal neutrophil recruitment during glycogen-induced peritonitis in rats were systematically defined.
Subjects: Male Long Evans rats (275–300 g).
Methods: Four hours after intraperitoneal injection of 25 mg oyster glycogen, neutrophilic exudates were harvested. Effects of blocking reagents (injected intravenously) to rat E-, L- and P-selectins, β_1 (VLA-4) and β_2 integrins (LFA-1 and Mac-1), ICAM-1, and the cytokines TNFα, IL-1 and IL-8 were assessed.
Results: Administration of synthetic sialyl Lewis^x oligosaccharide reduced neutrophil recruitment to the peritoneum by 26%. Antibody to E-selectin reduced neutrophil accumulation by 71%, while anti-L-selectin reduced neutrophil accumulation by 59%, and anti-P-selectin was without an effect. Similar patterns of inhibition were found when selectin-Ig chimeras were employed. Antibodies to LFA-1 (CD11a), Mac-1 (CD11b) or CD18 reduced neutrophil accumulation by 62%, 59% and 86%, respectively, while anti-VLA-4 was without effect. Anti-ICAM-1 reduced cell influx by 65%. IL-1 receptor antagonist and antibodies to IL-1 and human IL-8 reduced neutrophil accumulation by 43%, 40% and 62%, respectively. Unexpectedly, blockade of TNFα had no effect.
Conclusions: These studies identify requirements for selectins, β_2 integrins, IL-1 and a rat chemokine(s) similar to human IL-8 for neutrophil recruitment during glycogen-induced peritonitis. The lack of participation of VLA-4, P-selectin and TNFα suggests organ-specific cytokine and adhesion molecule requirements for neutrophil recruitment.

Key words: Peritonitis – Neutrophils – Inflammation – Cytokines – Rats

Correspondence to: P. A. Ward

Introduction

Neutrophil adhesion to the vascular endothelium and subsequent migration to an inflammatory site is a highly regulated process requiring the coordinated participation of cytokines (including chemokines) and leukocyte and endothelial adhesion molecules. Neutrophil recruitment into different organs often involves a repertoire of cytokines and adhesion molecules, and in some cases there are suggestions that there are tissue-specific roles for these mediators. Peritonitis induced by intraperitoneal injection of glycogen results in a large accumulation of neutrophils. It has been demonstrated that neutrophil recruitment under these conditions is dependent upon participation of L-selectin, since blockade of L-selectin with blocking antibody or L-selectin-Ig chimera reduces neutrophil accumulation [1, 2]. In P-selectin and/or ICAM-1 knock-out mice, these adhesion molecules have been described as participating in thioglycolate-induced peritoneal exudates [3, 4]. Others have suggested that neutrophil accumulation under these conditions requires a combination of both P- and E-selectin [5]. In other tissues such as lung, it has been shown that depending on the bacterial agent present, neutrophil accumulation may be CD18-dependent or independent [6]. Our own studies have demonstrated that neutrophil accumulation in lung following deposition of IgG immune complexes is dependent on CD11a, ICAM-1 and E-selectin, while CD11b plays a role in facilitating TNFα production by lung macrophages [7–9]. In lung, neutrophil accumulation also requires the participation of TNFα, IL-1, and IL-8 [10–12]. In contrast, in the dermis, neutrophil accumulation in response to vascular deposition of IgG immune complexes requires IL-1 but not TNFα [13]. In the current studies, we sought to further delineate tissue-specific mechanisms of neutrophil recruitment by systematically defining cytokine and adhesion molecule requirements in glycogen-induced peritonitis. Comparisons and contrasts to cytokine and adhesion molecules requirements in other organs of rats are noted.

Materials and methods

Glycogen-induced peritonitis

Oyster glycogen (Sigma, St. Louis, MO, USA) was dissolved in phosphate buffered saline (pH 7.4) to a final concentration of 1 mg/ml. A total of 25 ml were injected intraperitoneally into male Long Evans rats (275–300 g) and the peritoneal exudates were harvested 4 h later. Exudate cells were determined to be >95% neutrophils by microcytometry.

Synthetic sialyl Lewis^X oligosaccharides

Fucosylated and non-fucosylated versions of sialyl Lewis^x were employed in order to have a selectin-blocking intervention as described elsewhere [14, 15]. For selectin blockade, the fucosylated pentasaccharide version (SLX) was employed. As a negative control, the non-selectin-reactive, non-fucosylated form of SLX, sialyl-N-acetyllactosamine (SLN), was employed. The oligosaccharides were dissolved in PBS. A total of 200 μg was injected intraperitoneally with the glycogen preparation, followed by intravenous injections of 100 μg (in 0.5 ml) 2.5, 3.0 and 3.5 h after injection of glycogen. Using SLX, this dose schedule effectively blocks selectin-mediated lung neutrophil accumulation after intrapulmonary deposition of IgG immune complexes [15].

Selectin chimeras

The E-, L- and P-selectin-Ig chimeras were made in 293 cells and purified as described previously [2, 16]. The human CD4-Ig chimera was made as described by Capon et al. [17] and was used as an irrelevant control. The L-selectin-Ig chimera was of murine origin and the E- and P-selectin-Ig chimeras were of human origin [2,14]. A total of 2 mg E-, L-, or P-selectin-Ig chimera was injected intravenously 5 min before intraperitoneal injection of glycogen. When infused intravenously, none of the selectin chimeras cause neutropenia [18].

Blocking antibodies

The blocking antibodies and the times and routes of infusion were based on previous studies in which interventions were employed to block neutrophil accumulation in lung 4 hours after intrapulmonary deposition of IgG immune complexes [7–12]. Anti-rat VLA-4 (CD49d), clone TA-2, of the IgG_1 subclass, is reactive with the $\alpha_4$chain of β_1 integrin [19]. The antibody was infused intravenously in 0.25 ml (800 μg) just prior to glycogen injection. This protocol results in blocking of delayed-type hypersensitivity skin reactions in rats [20]. Anti-rat LFA-1 (CD11a), clone WT-1, of the murine IgG_{2a} subclass [21], and anti-rat Mac-1 (CD11b), clone 1B6c, of the murine IgG_1 subclass [22], were infused intravenously (200 μg) just prior to glycogen injection. Anti-rat CD18, clone CL26, of the IgG_1 subclass, was used as $F(ab')_2$ fragments as intact CL26 antibody has been shown to induce neutropenia [7]. Anti-rat ICAM-1, clone 1A29, of the IgG_1 subclass [23], was used as $F(ab')_2$ fragments, as intact 1A29 antibody does not block neutrophil accumulation in lung [7]. Anti-E-selectin, clone CL3, a murine monoclonal IgG_1 to human E-selectin with cross-reactivity to rat E-selectin was used as $F(ab')_2$ fragments, since intact CL3 neither blocked in vitro neutrophil adhesion to TNFα stimulated endothelial cells nor did it block neutrophil accumulation in rat lung [8]. Anti-rat L-selectin, clone HRL1, of the IgG_1 subclass, was prepared as described previously [24]. HRL1 was used as $F(ab')_2$ fragments since the intact antibody causes neutropenia [24]. Anti-P-selectin, clone PB1.3, a murine IgG_1 monoclonal antibody to human P-selectin with cross-reactivity to rat P-selectin [25], was injected intravenously (200 μg) just prior to glycogen injection. All antibodies used as $F(ab')_2$ fragments were administered intravenously (67 μg each) 2.5, 3.0 and 3.5 h after peritoneal instillation of glycogen. Antibodies to murine TNFα and IL-1 (goat polyclonal IgG) cross-reacted with rat IL-1 and TNFα [10, 11], and were infused intravenously (1.0 ml) as whole goat serum just prior to injection of glycogen. Murine monoclonal antibody (IgG_1) to human IL-8 (DM/C7) has been shown to be cross-reactive with a rat epitope and to suppress neutrophil influx into lung following intrapulmonary deposition of IgG immune complexes [12]. However, precisely what rat chemokine(s) DM/C7 reacts with has not been determined. A total of 200 μg DM/C7 were infused intravenously just prior to glycogen injection. IL-1 receptor antagonist (IL-1Ra) was a human recombinant protein shown to block binding of IL-1 to receptors on rat T-cells [26]. IL-1Ra (2 mg/kg) was injected subcutaneously just prior to glycogen injection. This antagonist has been shown to block IL-1 dependent lung inflammatory reactions in rats [13].

Statistical analyses

All values are expressed as mean ± SEM. Data were analyzed with a one-way analysis 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

Involvement of selectins for peritoneal recruitment of neutrophils

Roles for selectins in peritoneal neutrophil recruitment were provisionally evaluated using the P- and L-selectin-reactive oligosaccharide, SLX. Systemic administration of SLX reduced neutrophil numbers from $138 \pm 2.8 \times 10^6$ neutrophils in positive controls receiving the non-selectin-reactive oligosaccharide SLN, to $105 \pm 2.9 \times 10^6$ neutrophils, representing a 26% decrease ($p < 0.01$). To further delineate roles for E-, L- and P-selectins, blocking antibodies and selectin-Ig chimeras were employed. Blocking antibodies and chimeras to E-, L- and P-selectins were administered in dose schedules known to interfere with lung recruitment of neutrophils [8, 18, 24, 25]. Anti-E-selectin and anti-L-selectin reduced glycogen-induced peritoneal neutrophil accumulation by 71% and 59%, respectively, while anti-P-selectin had no effect (Fig. 1A). When selectin-Ig chimeras were used, similar inhibitory patterns were observed. Administration of E- or L-selectin-Ig's reduced peritoneal neutrophil accumulation by 33% and 50%, respectively, and P-selectin-Ig had no effect (Fig. 1B).

Involvement of β_1 and β_2 integrins and ICAM-1 for peritoneal neutrophil recruitment

Infusion of antibody to the rat β_1 integrin VLA-4 (CD49d) had no effect on peritoneal accumulation of neutrophils (Fig. 2A), even though this antibody is known to block the influx of mononuclear cells in delayed hypersensitivity reactions in rats [20]. Antibodies to the rat β_2 integrins LFA-1 (CD11a), Mac-1 (CD11b) and the β_2 common chain (CD18) reduced neutrophil accumulation by 62% , 59% and 86%, respectively (Fig. 2A). In view of the requirements for β_2 integrins, treatment with blocking antibody to the endothelial adhesion molecule ICAM-1 was employed in a

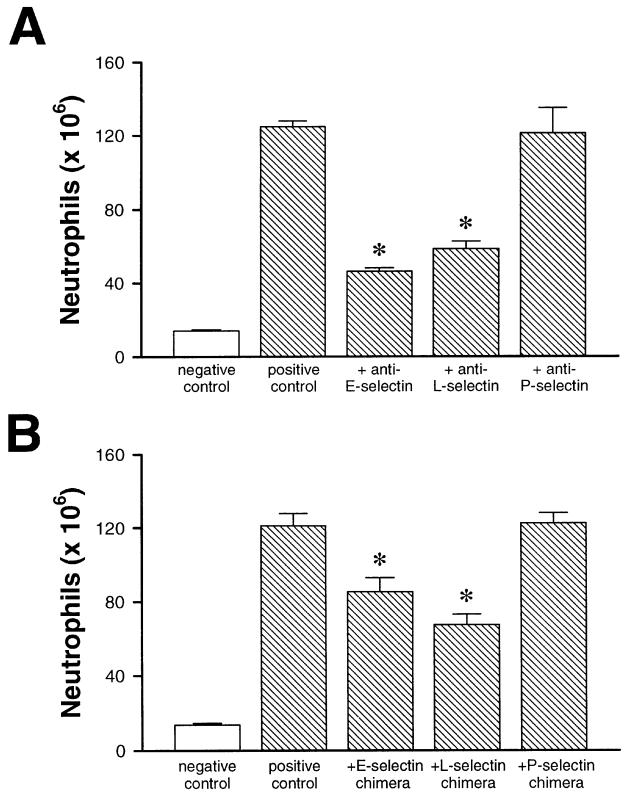

Fig. 1. Effects of selectin blockade on glycogen-induced peritoneal recruitment of neutrophils. Blocking antibodies (A), or selectin-Ig chimeras (B) were administered as indicated in Materials and methods. Negative controls (open bar) received 25 ml PBS i.p.; positive controls and treatment groups (hatched bars) received 25 mg oyster glycogen i.p. Negative and positive controls received either irrelevant IgG $F(ab')_2$ fragments (A), or CD4-chimera (B). For each group, $n = 4–8$. *$p < 0.01$ compared to positive control group.

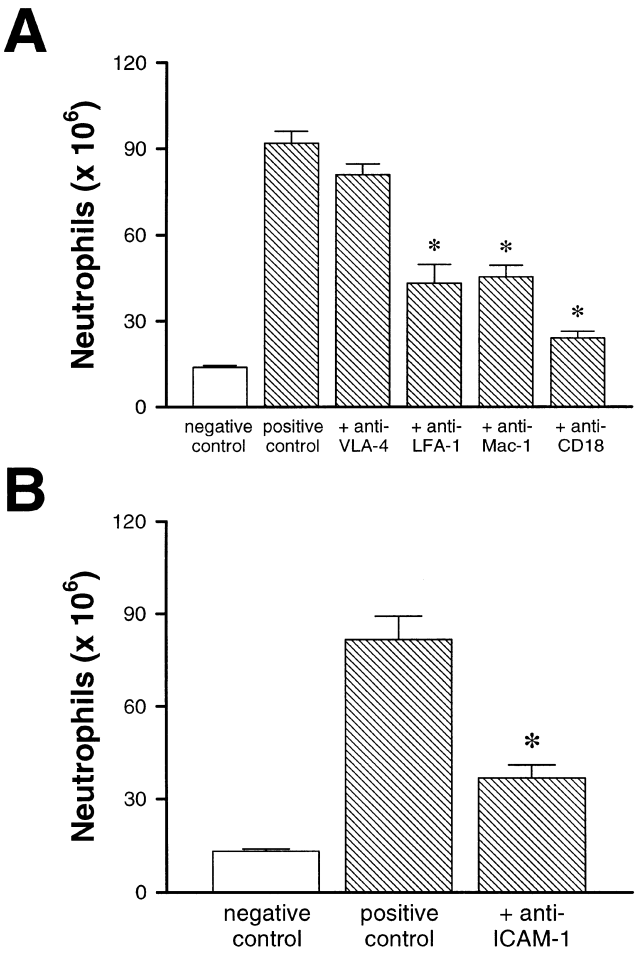

Fig. 2. Effects of blocking antibodies to β_1 and β_2 integrins (A), or ICAM-1 (B) on glycogen-induced peritoneal recruitment of neutrophils. Negative controls (open bar) received 25 ml PBS i.p.; positive controls and treatment groups (hatched bars) received 25 mg oyster glycogen i.p. Negative and positive controls received irrelevant IgG $F(ab')_2$ fragments. For each group, $n = 4–8$. *$p < 0.01$ compared to positive control group.

separate experiment. Consistent with inhibitory effects of β_2 integrin blockade, administration of $F(ab')_2$ anti-ICAM-1 reduced neutrophil accumulation by 65% (Fig. 2B).

Requirements for cytokines for peritoneal recruitment of neutrophils

Since endothelial cell upregulation of adhesion molecules by cytokines (i.e., TNFα, IL-1) is essential for lung accumulation of neutrophils following deposition of IgG immune complexes [27], roles of TNFα and IL-1 for peritoneal recruitment of neutrophils was examined using blocking antibodies or receptor antagonist. Unexpectedly, administration of antibody to TNFα had no effect on neutrophil recruitment to the peritoneum (Fig. 3). In contrast, antibodies to IL-1 reduced neutrophil accumulation by 40% (Fig. 3). The ability of anti-IL-1 to reduce glycogen-induced peritoneal accumulation of neutrophils was confirmed by treatment of rats with IL-1Ra. Administration of IL-1Ra reduced peritoneal neutrophil counts by 43%. To investigate a role of CXC chemokines in glycogen-induced peritoneal neutrophil recruitment, rats were treated with antibody to human IL-8. This antibody has been shown to react with a rat epitope and reduce lung accumulation of neutrophils in rats after IgG immune complex deposition [12]. Administration of anti-human IL-8 caused a 62% reduction in peritoneal neutrophil accumulation (Fig. 3). Thus, glycogen-induced peritoneal accumulation of rat neutrophils requires IL-1 and a rat homologue of IL-8, but not TNFα.

Discussion

These studies demonstrate that peritoneal recruitment of neutrophils during glycogen-induced peritonitis is dependent upon of E- and L-selectins (Fig. 1). The requirement for E-selectin implies that the presence of glycogen in the peritoneal cavity somehow results in upregulation of vascular E-selectin. In neutrophil-mediated lung injury induced by IgG immune complexes, lung TNFα and IL-1 production causes upregulation of pulmonary vascular adhesion molecules including ICAM-1 and E-selectin [8, 11, 27]. In the current studies, blockade of TNFα had no effect on neutrophil recruitment to the peritoneum, suggesting that IL-1 production may be linked to vascular upregulation of E-selectin and ICAM-1 in this model.

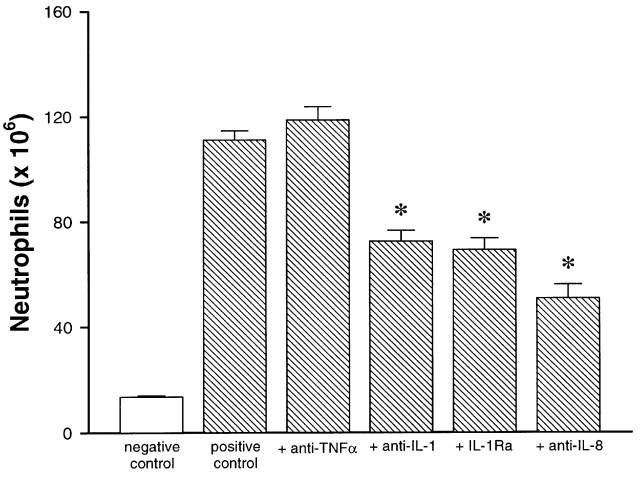

Fig. 3. Effects of cytokine blockade on glycogen-induced peritoneal recruitment of neutrophils. Negative controls (open bar) received 25 ml PBS i.p.; positive controls and treatment groups (hatched bars) received 25 mg oyster glycogen i.p. Negative and positive controls received irrelevant IgG antibody. For each group, n = 4–8. *p < 0.01 compared to positive control group.

Similarly, the requirement for L-selectin could be explained either by the fact that injection of glycogen leads to upregulation of a vascular endothelial ligand for L-selectin, or that L-selectin, through its SLX content, is reactive with endothelial E- and P-selectins, which has been suggested by other studies [28, 29]. However, a role for P-selectin was not identified in glycogen-induced peritoneal accumulation of neutrophils (Fig. 1). The failure to detect a role for P-selectin is not likely due to insufficient availability of blocking reagents, since both anti-P-selectin and P-selectin-Ig chimeric protein have relatively long half-lives in the circulation and in a model of lung injury induced by cobra venom factor in rats these treatment protocols efficiently reduce lung neutrophil accumulation [25]. Since P-selectin is transiently expressed on endothelial cells, a role for P-selectin in this model of inflammation may well have been missed because of the relatively long time course (4 h) for the full development of this inflammatory reaction. This is in contrast to mice lacking the gene encoding P-selectin, in which numbers of thioglycolate-induced peritoneal neutrophils have been reported to be reduced [3].

Selectin-mediated leukocyte adhesion is prerequisite for leukocyte integrin interactions with counter receptors on the vascular endothelium. Our data show that peritoneal recruitment of neutrophils is independent of the β_1 integrin VLA-4. This finding is not surprising, in as much as neutrophils express little, if any, VLA-4. However, neutrophils abundantly express the β_2 integrins, LFA-1 (CD11a) and Mac-1 (CD11b). The data described above indicate that both LFA-1 and Mac-1 are required for peritoneal accumulation of neutrophils. ICAM-1 is a primary endothelial counter-receptor for these β_2 integrins and blockade of ICAM-1 was also highly inhibitory for the accumulation of neutrophils. Endothelial cell expression of ICAM-1 is upregulated by proinflammatory cytokines including TNFα and IL-1 [27]. In the current model it appears that intraperitoneal production of IL-1, but not TNFα, contributes to upregulation of ICAM-1, increasing neutrophil adhesion and transmigration.

The requirement for IL-1 and exclusion of a role for TNFα in the peritoneal recruitment of neutrophils during glycogen-induced peritonitis is in contrast to neutrophil accumulation in lung following intrapulmonary deposition of IgG immune complexes which requires both TNFα and IL-1 [10, 11]. In the case of glomerular deposition of IgG immune complexes (in a model of acute nephrotoxic nephritis) in rats, this leads to an accumulation of neutrophils that requires TNFα but is independent of IL-1 [30]. The rat glomerular vasculature appears to be responsive to the renal arterial infusion of TNFα, resulting in the upregulation of glomerular adhesion molecules (E-selectin, ICAM-1, VCAM-1), but under similar conditions the infusion of IL-1 has little, if any, effect on the glomerular vasculature [30]. Accordingly, both the expression of TNFα and IL-1 as well as the vascular response to these cytokines appears to be variable, dependent on the vascular bed and the nature of the stimulus. The differential requirements for TNFα and IL-1 in the renal glomerular model are similar to those observed in other studies in which the recruitment of neutrophils into rat dermis following intradermal deposition of IgG immune complexes was shown to require IL-1 but not TNFα [13]. In those studies the lack of a role for TNFα appears to be related, not to the refractoriness of the dermal vasculature to TNFα, but rather, to the lack of intradermal expression of TNFα following vascular deposition of IgG immune complexes. It may be that rat macrophages or other cell types in skin, renal glomerulus and peritoneum can respond to stimuli with elaboration of IL-1, but not TNFα, in contrast to other areas such as lung. It seems likely that IL-1 is a key mediator in the peritoneal recruitment of neutrophils through upregulation of E-selectin and ICAM-1.

Finally, the ability of anti-human IL-8 to reduce neutrophil accumulation during glycogen-induced peritonitis is similar to previous studies in which the same antibody substantially reduced neutrophil influx into dermal or pulmonary inflammatory sites [12, 31]. Our current data suggest that antibody to human IL-8 (DM/C7) cross-reacts with a rat product that has functional role as a proinflammatory mediator required for peritoneal neutrophil recruitment as in lung. The DM/C7 antibody is reactive with a rat epitope that appears to be homologous to human IL-8, although our attempts to identify this product by immunoaffinity techniques have, to date, been unsuccessful. Notwithstanding, our data suggest that an IL-8-like CXC chemokine plays a central role in the recruitment of neutrophils during peritoneal inflammation.

In summary, the data in this report identify adhesion molecule and cytokine requirements for neutrophil recruitment during glycogen-induced peritonitis. Accumulation of neutrophils in the peritoneum appears to be dependent upon L-selectin and the β_2 integrins LFA-1 and Mac-1 on neutrophils, and E-selectin and ICAM-1 on the endothelium. Additionally, neutrophil recruitment to the peritoneum requires the participation of IL-1 and an IL-8-like molecule, but not TNFα. Thus, these findings demonstrate important organ-specific roles for cytokines and adhesion molecules in the in vivo recruitment of neutrophils.

Acknowledgments. The authors gratefully acknowledge the generous gifts of selectin-Ig chimeras from Dr. Susan Watson, Genentech Corp., S. San Francisco, CA, USA; anti-rat CD11b from Dr. Robert Todd III

(Department of Internal Medicine, University of Michigan Medical School); anti-IL-8 (DM/C7) from Dr. Mark Bolanowski (The Monsanto Company, St. Louis, MO, USA); antibody to rat VLA-4 from Dr. Thomas Issekutz (Department of Pathology, Dalhousie University Medical School, Halifax, Nova Scotia, Canada); and oligosaccharides from Dr. James C. Paulson (Cytel Corporation, San Diego, CA, USA).

References

[1] Jutila MA, Rott L, Berg EL, Butcher EC. Function and regulation of the neutrophil MEL-14 antigen in vivo: Comparison with LFA-1 and MAC-1. J Immunol 1989;143:3310–8.

[2] Watson SR, Fennie C, Lasky LA. Neutrophil influx into an inflammatory site inhibited by a soluble homing receptor-IgG chimera. Nature 1991;349:164–7.

[3] Hynes RO, Wagner DD. Genetic manipulation of vascular adhesion molecules in mice. J Clin Invest 1996;98:2193–5.

[4] Bullard DC, Qin L, Lorenzo I, Quinlan WM, Doyle NA, Vestweber D, et al. P-selectin/ICAM-1 double mutant mice: Acute emigration of neutrophils into the peritoneum is completely absent but is normal into pulmonary alveoli. J Clin Invest 1995;95:1782–8.

[5] Ramos CL, Kunkel EJ, Lawrence MB, Jung U, Vestweber D, Bosse R, et al. Differential effect of of E-selectin antibodies on neutrophil rolling and recruitment to inflammatory sites. Blood 1997;89:3009–18.

[6] Doerschuk CM, Winn RK, Coxson HO, Harlan JM. CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. J Immunol 1990;144:2320–7.

[7] Mulligan MS, Wilson GP, Todd RF, Smith CW, Anderson DC, Varani J, et al. Role of β_1, β_2 integrins and ICAM-1 in lung injury following deposition of IgG and IgA immune complexes. J Immunol 1993;150:2407–17.

[8] Mulligan MS, Varani J, Dame MK, Lane CL, Smith CW, Anderson DC, et al. Role of endothelial-leukocyte adhesion molecule 1 (ELAM-1) in neutrophil-mediated lung injury in rats. J Clin Invest 1991;88:1396–406.

[9] Mulligan MS, Vaporciyan AA, Warner RL, Jones ML, Foreman KE, Miyasaka M, et al. Compartmentalized roles for leukocytic adhesion molecules in lung inflammatory injury. J Immunol 1995;154:1350–63.

[10] Warren JS, Yabroff KR, Remick DG, Kunkel SL, Chensue SW, Kunkel RG, et al. Tumor necrosis factor participates in the pathogenesis of acute immune complex alveolitis in the rat. J Clin Invest 1989;84:1873–82.

[11] Warren JS. Intrapulmonary interleukin 1 mediates acute immune complex alveolitis in the rat. Biochem Biophys Res Commun 1991;175:604–10.

[12] Mulligan MS, Jones ML, Bolanowski MA, Baganoff MP, Deppeler CL, Meyers DM, et al. Inhibition of lung inflammatory reactions in rats by an anti-human IL-8 antibody. J Immunol 1993;150:5585–95.

[13] Mulligan MS, Ward PA. Immune complex-induced lung and dermal vascular injury. Differing requirements for tumor necrosis factor-alpha and IL-1. J Immunol 1992;149:331–9.

[14] Mulligan MS, Paulson JC, De Frees S, Zheng ZL, Lowe JB, Ward PA. Protective effects of oligosaccharides in P-selectin-dependent lung injury. Nature 1993;364:149–51.

[15] Mulligan MS, Lowe JB, Larsen RD, Paulson J, Zheng Z, DeFrees S, et al. Protective effects of sialylated oligosaccharides in immune complex-induced acute lung injury. J Exp Med 1993;178:623–31.

[16] Erbe DV, Watson SR, Presta LG, Wolitzky BA, Foxall C, Brandley BK, et al. P- and E-selectin use common sites for carbohydrate ligand recognition and cell adhesion. J Cell Biol 1993;120:1227–32.

[17] Capon DJ, Chamow SM, Mordenti J, Marsters SA, Gregory T, Mitsuya H, et al. Designing CD4 immunoadhesions for AIDS therapy. Nature 1989;337:525–31.

[18] Mulligan MS, Watson SR, Fennie C, Ward PA. Protective effects of selectin chimeras in neutrophil-mediated lung injury. J Immunol 1993;151:6410–7.

[19] Issekutz TB, Wykretowicz A. Effect of a new monoclonal antibody, TA-2, that inhibits lymphocyte adherence to cytokine stimulated endothelium in the rat. J Immunol 1991;147:109–16.

[20] Issekutz TB. Inhibition of in vivo lymphocyte migration to inflammation and homing to lymphoid tissues by the TA-2 monoclonal antibody: A likely role for VLA-4 in vivo. J Immunol 1991; 147:4178–84.

[21] Tamatani TM, Kotani T, Miyasaka M. Characterization of the rat leukocyte integrin, CD11/CD18, by the use of LFA-1 subunit-specific monoclonal antibodies. Eur J Immunol 1991; 21:627–33.

[22] Mulligan MS, Smith CW, Anderson DC, Todd RF, Miyasaka M, Tamatani T, et al. Role of leukocyte adhesion molecules in complement-induced lung injury. J Immunol 1993;150;2401–6.

[23] Tamatani TM, Miyasaka M. Identification of monoclonal antibodies reactive with the rat homologue of ICAM-1 and evidence for differential involvement of ICAM-1 in the adherence of resting versus activated lymphocytes to high endothelial cells. Int Immunol 1990;2:166–72.

[24] Mulligan MS, Miyasaka M, Tamatani T, Jones ML, Ward PA. Requirements for L-selectin in neutrophil-mediated lung injury in rats. J Immunol 1994;152:832–40.

[25] Mulligan MS, Polley MJ, Bayer RJ, Nunn MF, Paulson JC, Ward PA. Neutrophil-dependent acute lung injury. Requirement for P-selectin (GMP-140). J Clin Invest 1992;90:1600–7.

[26] Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature (Lond) 1990;343:336–40.

[27] Mulligan MS, Vaporciyan AA, Miyasaka M, Tamatani T, Ward PA. Tumor necrosis factor alpha regulates in vivo intrapulmonary expression of ICAM-1. Am J Pathol 1993;142:1739–49.

[28] Polley MJ, Phillips ML, Wayner E, Nudelman E, Singhal AK, Hakomori S, et al. CD62 and endothelial cell-leukocyte adhesion molecule 1 (ELAM-1) recognize the same carbohydrate ligand, sialyl-Lewis^x. Proc Natl Acad Sci USA 1991;88:6224–8.

[29] Picker LJ, Warnock RA, Burns AR, Doerschuk CM, Berg EL, Butcher EC. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. Cell 1991;66:921–33.

[30] Mulligan MS, Johnson KJ, Todd RF, Issekutz TB, Miyasaka M, Tamatani T, et al. Requirements for leukocyte adhesion molecules in nephrotoxic nephritis. J Clin Invest 1993;91:577–87.

[31] Zhang Y, Ramos BF, Jakschik B, Baganoff MP, Deppeler CL, Meyer DM, et al. Interleukin 8 and mast cell-generated tumor necrosis factor-alpha in neutrophil recruitment. Inflammation 1995;19:119–32.