

Role of Selectins in the Intestinal Epithelial Migration of Eosinophils

SONIA MICHAIL, ETHAN MEZOFF, AND FRANK ABERNATHY

Department of Pediatrics, Division of Gastroenterology and Nutrition and Wright State University School of Medicine and The Children's Medical Center, One Children's Plaza, Dayton, OH 45404

ABSTRACT

Understanding the mechanisms by which eosinophils migrate into and across the intestinal epithelium can provide alternative therapeutic targets for conditions characterized by eosinophilic cryptitis and crypt abscesses. Eosinophil migration is dependent on adhesion molecules such as selectins. Human eosinophils express L-selectin and P-selectin counterligand P-selectin glycoprotein ligand-1 (PSGL-1). The tetrasaccharide sialyl Lewis^x (sLe^x) binds to all three selectins, so compounds that mimic sLe^x, such as TBC1269, are potential antagonists. We hypothesized that eosinophils migrate from the basolateral to the apical surface of intestinal epithelium through the orchestrated effects of selectins. TBC1269 was added to fluorescently labeled HL-60 clone 15 eosinophils as well as human blood eosinophils, in incremental amounts. Subsequently, blocking antibodies toward L-selectin and PSGL-1 were used in a similar manner. HL-60 eosinophils were allowed to migrate into T-84 monolayers. The number of migrated HL-60 cells was calculated by comparing fluorescence with known cell densities. HL-60 and human eosinophils that

were undergoing migration were significantly lower in the presence of TBC1269. This effect was concentration dependent, and near complete inhibition of migration was seen at a TBC1269 concentration of 10 mg/mL. In addition, HL-60 eosinophil migration was significantly lower in the presence of the blocking antibodies to PSGL-1 and L-selectin (39.2 and 51.6% inhibition, respectively). Simultaneous blocking of PSGL-1 and L-selectin resulted in inhibition of 76.0% of the migration. The results of this study suggest a major role for selectins in the intestinal epithelial migration of differentiated eosinophils. sLe^x, L-selectin, and the P-selectin counterligand PSGL-1 can be potential therapeutic targets. (*Pediatr Res* 58: 644–647, 2005)

Abbreviations

fMLP, N-formyl methionine leucine phenyl alanine
PSGL-1, P-selectin glycoprotein ligand-1
sLe^x, tetrasaccharide sialyl Lewis^x

The gastrointestinal tract represents the largest reservoir of eosinophils within the body. In the gut, eosinophils play an important role in several inflammatory disorders (1). During these conditions, the eosinophils migrate into the intestinal lumen, forming eosinophilic crypt abscesses (2). Eosinophil products then are released, which can contribute to epithelial damage (3).

The infiltration of the leukocytes to the areas of injury and inflammation is accomplished by the coordinated actions of cellular adhesion molecules under the direction of chemokine signaling. Three families of adhesion molecules contribute to eosinophil migration: selectins, integrins, and immunoglobulins. The family of selectins consists of three members: L-, P-, and E-selectins. The interaction between the selectins and their ligands is carbohydrate based. Therefore, compounds such as

the tetrasaccharide sialyl Lewis^x (sLe^x) are known to bind to all selectins, acting as an antagonist (4).

The process of eosinophil migration across the endothelium has been widely investigated, yet little is known about eosinophil–epithelial interactions in the gut. Measures that can prevent eosinophil recruitment into the intestinal epithelium are likely to have therapeutic value. Primary molecular targets for prevention of eosinophil accumulation are the adhesion molecules that mediate leukocyte interactions with vascular endothelium and extracellular matrix proteins.

However, studies of eosinophil function are hindered by the relative paucity of eosinophils in the circulation and the difficulty in purification from neutrophils. Furthermore, eosinophils that are isolated from different individuals may respond differently to identical conditions. Thus, HL-60–differentiated eosinophils present a considerable advantage in large-scale studies for eosinophil chemotaxis. Previous studies in our laboratory demonstrated that HL-60 clone 15 cells function as a viable model for the study of eosinophil migration across intestinal epithelial monolayers (5).

Received October 14, 2004; accepted January 26, 2005.

Correspondence: Sonia Michail, M.D., One Children's Plaza, Dayton, OH 45404; e-mail: sonia.michail@wright.edu.

This work was supported in part by the Wright State University School of Medicine.

DOI: 10.1203/01.PDR.0000180572.65751.F4

We hypothesized that eosinophils migrate from the basolateral to the apical surface of intestinal epithelium through the orchestrated effects of selectins. This study examined the inhibitory effect of sLe^x mimetic on the intestinal epithelial migration of HL-60–differentiated eosinophils, as well as human peripheral eosinophils. Consequently, the role of individual selectins during the process of migration was evaluated. The results of this study suggest a major role for selectins in the intestinal epithelial migration of differentiated eosinophils.

METHODS

Supplies were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

Preparation of HL-60 eosinophils. One week before chemotactic experiments, clone 15 HL-60 cells [American Tissue Culture Collection (ATCC), Rockville, MD], maintained in alkaline medium, were suspended at 2×10^5 cells/mL in RPMI medium (Life Technologies, Gaithersburg, MD) that contained 0.5 mM butyric acid and 5 ng/mL GM-CSF (R&D Systems, Minneapolis, MN) to allow the HL-60 cells to differentiate into eosinophils. Cell culture was continued for 7 d without change of the medium. Consistent with what other investigators have shown, the cell viability was >98% when assessed by 0.2% trypan blue dye exclusion. To assess purity of eosinophilic differentiation, cells were stained with Luxol fast blue. As with other reported experience, cell purity was >90% (6,7).

Preparation of monolayers. T-84 cells (ATCC) were maintained in DMEM/F12 (Life Technologies), fetal bovine serum (Atlanta Biologicals, Atlanta, GA), penicillin, streptomycin, and amphotericin (Life Technologies) in a humidified atmosphere with 5% CO₂. Approximately 100×10^3 cells were plated on the undersurface of each fluoroblock cell culture insert as previously described (5) (Fisher Scientific, Pittsburgh, PA). The cells were allowed to become confluent, an average of 8–10 d after plating. Confluent density was $\sim 5 \times 10^5$ cells/cm².

Chemotaxis assay. Cell tracker green (5-chloromethyl fluorescein; Molecular Probes, Eugene, OR) fluorescent dye was used to label the HL-60 clone 15 eosinophils. The fluorescent dye (5–10 μ M) was incubated with the cells for 15 min at room temperature with continuous mixing. HL-60 cells were washed once in RPMI to remove any excessive soluble dye, then allowed to incubate for another 30 min at 37°C in medium without dye. Tubes were gently shaken every 10 min. The cells (1.0×10^6) then were allowed to incubate for 150 min on inverted and confluent T-84 monolayers separated by a 0.3-cm² fluoroblock insert membrane with 8- μ pores. N-formyl methionine leucine phenyl alanine (fMLP) was used as a chemoattractant. Relative fluorescent units were read by the Genios fluorometer (Tecan US Inc., Research Triangle Park, NC). Relative fluorescent units of migrated cells were converted to cell numbers on the basis of comparisons with known densities of HL-60 cells. Control monolayers underwent the same procedure without being exposed to the chemoattractant agent.

TBC1269 and fMLP-induced HL-60 migration. fMLP was added to the apical bathing solution at a concentration of 1 μ M to determine its effect on the migration of HL-60 eosinophils. Migration was determined as described above. TBC1269 (Texas Biotechnology, Houston, TX), which is a sLe^x mimetic with structural similarities to sialyl dimeric-Lewis^x that has been shown to inhibit selectins and their ligands resulting in the inhibition of the adhesion of leukocytes and their recruitment to sites of injury *in vivo* and *in vitro* (8,9), was added to HL-60 clone 15 cells in incremental amounts for 30 min before migration. Migration was allowed to proceed and was quantified as described above.

L-selectin and P-selectin glycoprotein ligand-1 blockade and fMLP-induced HL-60 eosinophil migration. To determine the role of individual selectins and their ligands, blocking antibodies were used. The blocking antibodies were targeted against the selectins and the selectin ligands, which are known to be expressed by eosinophils, namely L-selectin and the P-selectin glycoprotein ligand-1 (PSGL-1). Blocking antibodies at 1–10 μ g/mL (6.7–67 nM) and 50–100 μ g/mL (13.5–27 nM), respectively, were allowed to incubate with the HL-60 cells for 30 min before the migration experiment. Such concentration of the blocking antibodies is similar to those used in previously published reports (10). Migration then was allowed to proceed as described above.

Effect of TBC1269 on the intestinal epithelial migration of human peripheral eosinophils. Human peripheral eosinophils were used to validate the migration experiments performed with the HL-60 eosinophils. Briefly,

blood was obtained from human adult donors in accordance with a protocol approved by the Institution Review Board.

Eosinophils were isolated by centrifuging dextran-sedimented leukocytes on Ficoll-Histopaque (Sigma Chemical Co.). The leukocytes that were isolated were washed in PBS without calcium or magnesium. The eosinophils were purified from the neutrophils by immunomagnetic removal (MACS system; Miltenyi Biotec Systems, Auburn, CA) of CD16-positive cells (neutrophils). The isolated human eosinophils have a viability and purity >95%. The pan-selectin antagonist, TBC1269, was added in incremental amounts to the eosinophils for 30 min before migration. The number of migrating eosinophils was determined as described above.

RESULTS

Effect of TBC1269 on the migration of HL-60–differentiated eosinophils. TBC1269, which acts as a pan-selectin antagonist, resulted in the inhibition of the intestinal epithelial migration of HL-60–differentiated eosinophils. The effect was dose dependent, and complete inhibition of migration was seen at 10 mg/mL concentration (Fig. 1). Therefore, the migration of HL-60–differentiated eosinophils across intestinal epithelial migration seems to be dependent on selectins and/or their ligands. The individual selectins/ligands then were investigated as potential players in the process of migration as described below.

TBC at 10 mg/mL (11 mM) concentration was not found to have any toxic effect on the function or viability of the intestinal epithelial monolayer or the differentiated eosinophils. The following validating experiments were conducted: differentiated eosinophils were incubated with TBC1269 at 10 mg/mL concentration for 30 min, followed by a 150-min incubation in the top reservoir in accordance with the original protocol. The trans-epithelial electrical resistance at the conclusion of the study was measured. The viability of the differentiated eosinophils and T-84 cells, in the presence or absence of TBC1269, was assayed by Trypan blue exclusion. The viability and function of the T-84 cells were comparable to the control cells (viability 88 and 87% for control and TBC, respectively; $n = 3$; no decrease in TEER was seen in any of the monolayers). The viability of the differentiated eosinophils was similar in the presence or absence of TBC1269 (96 and 97% for control and TBC, respectively; $n = 3$).

Effect of L-selectin and PSGL-1 blockade on the migration of HL-60–differentiated eosinophils. Anti-L-selectin MAb was incubated with the HL-60 cells for 30 min before migration.

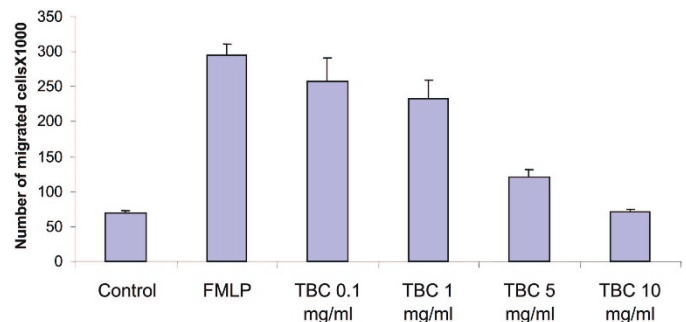


Figure 1. Dose-response effect of TBC on HL-60 eosinophil migration. The addition of the pan-selectin antagonist TBC 1269 to HL-60 eosinophils resulted in the inhibition of their migration in a concentration-dependent manner. Almost total inhibition is seen at a concentration of 10 mg/mL ($p < 0.05$; $n = 9$).

tion. Inhibitory effects were seen at 1 and 10 $\mu\text{g/mL}$, representing an average of 39.2% inhibition when compared with the fMLP-induced positive control. Anti-PSGL-1 MAb was incubated with HL-60 cells for 30 min before migration. Inhibitory effects were seen at 50 and 100 $\mu\text{g/mL}$ concentrations, representing an average of 51.6% inhibition (Fig. 2). When the combination of both blocking antibodies for L-selectin (10 $\mu\text{g/mL}$) and PSGL-1 (100 $\mu\text{g/mL}$) were added to HL-60 eosinophils, the migration process was further inhibited by 76.0% (Fig. 2).

Effect of TBC1269 on the migration of human peripheral eosinophils. For validating the TBC1269 effect on the migration of the human peripheral eosinophils across a fMLP gradient, TBC1269 was incubated with human peripheral eosinophils before migration into the intestinal epithelial monolayer. TBC1269 inhibited the migration of the eosinophils in a concentration-dependent manner (Fig. 3).

DISCUSSION

Eosinophils migrate into the intestinal epithelium during several eosinophilic inflammatory conditions (1). Migration into the epithelium is comparable to the development of eosinophilic cryptitis, whereas migration across the intestinal epithelium is comparable to the development of crypt abscess. Both conditions are the hallmark of a variety of disorders now known as eosinophilic gastrointestinal disorders (11,12). Disease development is clearly associated with the migration of eosinophils. Other conditions characterized by the presence of eosinophilic cryptitis and crypt abscess include radiation proctitis, protein-sensitive enteropathy, and helminthic infections (1,13–15). Evidence is mounting to suggest a potential role for eosinophils in inflammatory bowel disease (16–23). During the eosinophilic diseases of the intestine, the eosinophils migrate into the intestinal lumen, forming what is known as eosinophilic crypt abscesses (24). Eosinophil toxic products, such as cationic proteins, oxygen metabolites, proteases, and lipid

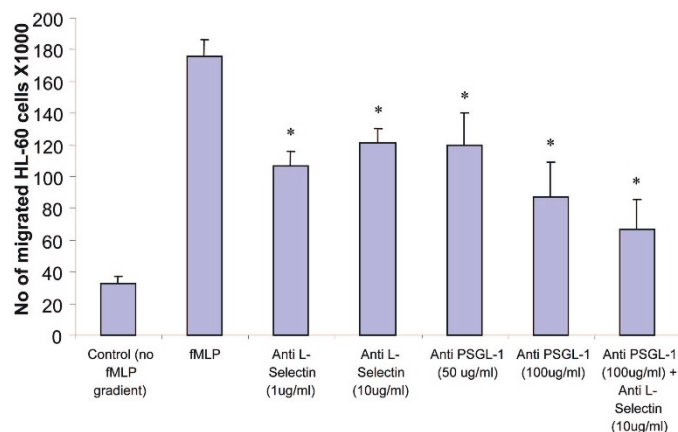


Figure 2. Selectin blockade and its effect on intestinal migration of HL-60 eosinophils. Blocking L-selectin and PSGL-1 inhibits the migration of HL-60–differentiated eosinophils. Blocking both L-selectin and PSGL-1 results in further inhibition of migration compared with the individual blocking effects [*groups with statistically significant ($p < 0.05$; $n = 9$) inhibition of migration when compared with the uninhibited fMLP-induced migration].

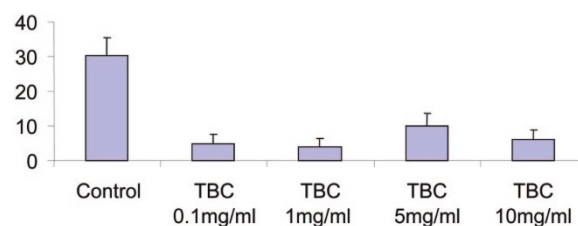


Figure 3. The effect of TBC1269 on the migration of human peripheral eosinophils. The addition of the pan-selectin antagonist TBC1269 to human peripheral eosinophils resulted in the blocking of the fMLP-induced intestinal epithelial migration ($p < 0.05$ for all groups; $n = 6$; migration reported as number of eosinophils $\times 10^3$).

mediators, are released, which can contribute to epithelial damage (25).

The infiltration of the leukocytes to the areas of injury and inflammation is accomplished by the coordinated actions of cellular adhesion molecules under the direction of chemokine signaling. A variety of cell adhesion molecules are expressed and used by many leukocytes. Three major groups are involved: selectins, integrins, and the immunoglobulin gene superfamily. P- and E-selectin mediate initial leukocyte adhesion, whereas $\beta 2$ -integrin/intercellular adhesion molecule-1 and VLA-4/vascular cellular adhesion molecule-1 (Very Late Antigen) pathways mediate leukocyte arrest and transendothelial migration. The process of eosinophil migration across the endothelium has been widely investigated, yet little is known about eosinophil-epithelial interactions in the gut. Measures that can prevent eosinophil recruitment into and across the intestinal epithelium may have therapeutic value. Primary molecular targets for prevention of eosinophil accumulation are the adhesion molecules that mediate leukocyte interactions with vascular endothelium and extracellular matrix proteins.

However, studies of eosinophil function are hindered by the relative paucity of eosinophils in the circulation and the difficulty in purification from neutrophils. In our studies (26,27), ~18–20 million eosinophils are needed to run an individual experiment. Such numbers are difficult to obtain from volunteers. Thus, cell lines that can differentiate into eosinophils present a considerable advantage in large-scale studies for eosinophil chemotaxis. HL-60 is one of several human leukemic cell lines that can be induced to differentiate into cells with histologic, biochemical, and functional characteristics of mature human eosinophils. Specific clones of HL-60, such as clone 15, yield a high concentration of eosinophils when treated with butyric acid (6,7). HL-60 cells develop proteins, enzymes, and adhesion molecules characteristic of human eosinophils (28–30). They are known to express L-selectin as well (31,32).

Integrins that are expressed on eosinophils bind to adhesion receptors that belong to the immunoglobulin superfamily on endothelial cells and are implicated in the subsequent firmer adhesion (33). Other adhesion molecules that are involved in leukocyte migration include the immunoglobulin superfamily. The three known selectin members are E-selectin, L-selectin, and P-selectin, which are also referred to as CD62 followed by their respective first letters (CD62E, CD62L, and CD62P). E-selectin is expressed on activated endothelium. P-selectin (34), the largest selectin, originally received its name because

of its stimulus-dependent expression on platelets, but it is also rapidly and transiently expressed on other cells, such as endothelial cells. L-selectin is the smallest selectin and receives its name because its expression is restricted to leukocytes. Eosinophils constitutively express L-selectin, and they shed it on cell activation (35). Eosinophils use a functional epitope of L-selectin for ligand binding different from neutrophils (36,37). The counterligands for P-selectin on eosinophils is PSGL-1 (38). sLe^x binds to the three known selectins; therefore, compounds that inhibit sLe^x act as a selectin antagonist. TBC1269 is a nonoligosaccharide sLe^x antagonist and has been shown to block binding to all selectins (39,40). In general, eosinophils have been shown in other studies, as well as the current, to require much more TBC1269 to produce an inhibitory effect compared with the neutrophils (40). In this model of intestinal migration of eosinophils, TBC1269 compound displays potent inhibitory effect, resulting in almost complete abolishment of the migration process. This effect is demonstrated when both HL-60–differentiated eosinophils and human peripheral eosinophils are used.

The results of this study support a novel function for the selectins. Selectins are traditionally known to be involved in the initial tethering and rolling of leukocytes while the cells are under flow conditions. However, our complete understanding of the selectin role and function is a goal yet to be reached. This study demonstrates a role for the selectins under steady-state conditions and opens avenues to be explored in the future.

TBC1269 selectin antagonism does not distinguish the role of an individual selectin during the process of migration. Therefore, the role of L-selectin and PSGL-1 were individually studied using function-blocking antibodies. Both L-selectin and PSGL-1 play a role during the process of migration. However, blocking their effect still does not result in absolute inhibition of migration as seen with TBC1269. Other carbohydrate-based adhesion molecules or yet-to-be discovered selectins may play a role in the process of migration as well. Overall, the results of this study suggest an important role for selectins in the intestinal epithelial migration of differentiated eosinophils. sLe^x, L-selectin, and the P-selectin counterligand PSGL-1 may prove useful as potential therapeutic targets.

REFERENCES

1. Rothenberg ME, Mishra A, Brandt EB, Hogan SP 2001 Gastrointestinal eosinophils in health and disease. *Adv Immunol* 78:291–328
2. Blackshaw AJ, Levison DA 1986 Eosinophilic infiltrates of the gastrointestinal tract. *J Clin Pathol* 39:1–7
3. Gleich GJ, Adolphson CR, Leiferman KM 1993 The biology of the eosinophilic leukocyte. *Annu Rev Med* 44:85–101
4. Foxall C, Watson SR, Dowbenko D, Fennie C, Lasky LA, Kiso M, Hasegawa A, Asa D, Brandley BK 1992 Three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. *J Cell Biol* 117:895–902
5. Michail S, Abernathy F 2004 A new model for studying eosinophil migration across cultured intestinal epithelial monolayers. *J Pediatr Gastroenterol Nutr* 39:56–63
6. Fischkoff SA, Pollak A, Gleich GJ, Testa JR, Misawa S, Reber TJ 1984 Eosinophilic differentiation of the human promyelocytic leukemia cell line, HL-60. *J Exp Med* 160:179–196
7. Fischkoff SA 1988 Graded increase in probability of eosinophilic differentiation of HL-60 promyelocytic leukemia cells induced by culture under alkaline conditions. *Leuk Res* 12:679–686
8. Rao BN, Anderson MB, Musser JH, Gilbert JH, Schaefer M, Foxall C, Brandley BK 1994 Sialyl Lewis X mimics derived from a pharmacophore search are selectin inhibitors with anti-inflammatory activity. *J Biol Chem* 269:19663–19666
9. Palma-Vargas JM, Toledo-Pereyra L, Dean RE, Harkema JM, Dixon RA, Kogan TP 1997 Small-molecule selectin inhibitor protects against liver inflammatory response after ischemia and reperfusion. *J Am Coll Surg* 185:365–372
10. Walcheck B, Leppanen A, Cummings RD, Knibbs RN, Stoolman LM, Alexander R, Mattila PE, McEver RP 2002 The monoclonal antibody CHO-131 binds to a core 2 O-glycan terminated with sialyl-Lewis x, which is a functional glycan ligand for P-selectin. *Blood* 99:4063–4069
11. Rothenberg ME 2004 Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 113:11–28
12. Kelly KJ 2000 Eosinophilic gastroenteritis. *J Pediatr Gastroenterol Nutr* 30(suppl):S28–S35
13. Goldblum JR, Crawford JM 2004 Surgical Pathology of the Gastrointestinal Tract, Liver, Biliary Tree and Pancreas, 1st Ed. WB Saunders Company, Philadelphia, pp 191–192, 234–235
14. Sampson HA 2004 Update on food allergy. *J Allergy Clin Immunol* 113:805–819
15. Schwab D, Muller S, Aigner T, Neureiter D, Kirchner T, Hahn EG, Raithel M 2000 Functional and morphologic characterization of eosinophils in the lower intestinal mucosa of patients with food allergy. *Am J Gastroenterol* 98:1525–1534
16. Winterkamp S, Raithel M, Hahn EG 2000 Secretion and tissue content of eosinophil cationic protein in Crohn's disease. *J Clin Gastroenterol* 30:170–175
17. Bischoff SC, Wedemeyer J, Herrmann A, Meier PN, Trautwein C, Cetin Y, Maschek H, Stolte M, Gebel M, Manns MP 1996 Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. *Histopathology* 28:1–13
18. Dubucquoi S, Janin A, Klein O, Desreumaux P, Quandalle P, Cortot A, Capron M, Colombel JF 1995 Activated eosinophils and interleukin 5 expression in early recurrence of Crohn's disease. *Gut* 37:242–246
19. Jeziorska M, Haboubi N, Schofield P, Woolley DE 2001 Distribution and activation of eosinophils in inflammatory bowel disease using an improved immunohistochemical technique. *J Pathol* 194:484–492
20. Hankard GF, Brousse N, Cezard JP, Emillie D, Peuchmaur M 1997 In situ interleukin 5 gene expression in pediatric Crohn's disease. *J Pediatr Gastroenterol Nutr* 24:568–572
21. Saitoh O, Kojima K, Sugi K, Matsuse R, Uchida K, Tabata K, Nakagawa K, Kayazawa M, Hirata I, Katsu K 1999 Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. *Am J Gastroenterol* 94:3513–3520
22. Lampinen M, Carlson M, Sangfelt P, Taha Y, Thorn M, Loof L, Raab Y, Venge P 2001 IL5 and TNF- α participate in recruitment of eosinophils to intestinal mucosa in ulcerative colitis. *Dig Dis Sci* 46:2004–2009
23. Desreumaux P, Nutten S, Colombel JF 1999 Activated eosinophils in inflammatory bowel disease: do they matter? *Am J Gastroenterol* 94:3396–3398
24. Blackshaw AJ, Levison DA 1986 Eosinophilic infiltrates of the gastrointestinal tract. *J Clin Pathol* 39:1–7
25. Gleich GJ, Adolphson CR, Leiferman KM 1993 The biology of the eosinophilic leukocyte. *Annu Rev Med* 44:85–101
26. Michail S, Abernathy F 2002 Transepithelial migration of HL-60 differentiated eosinophils is induced by enteropathogenic *Escherichia coli* infection. *J Pediatr Gastroenterol Nutr* 35:423
27. Michail S, Abernathy F 2002 A novel model for studying transepithelial migration of eosinophils across a cultured intestinal epithelium. *Gastroenterology* 122:A151
28. Fischkoff SA, Brown GE, Pollak A 1986 Synthesis of eosinophil-associated enzymes in HL-60 promyelocytic leukemia cells. *Blood* 68:85–192
29. Hua J, Hasebe T, Someya A, Nakamura S, Sugimoto K, Nagaoka I 2001 Evaluation of the expression of NADPH oxidase components during maturation of HL-60 clone 15 cells to eosinophilic lineage. *Inflamm Res* 50:156–167
30. Lundahl J, Sehmi R, Hayes L, Howie K, Denburg JA 2000 Selective upregulation of a functional $\beta 7$ integrin on differentiating eosinophils. *Allergy* 55:865–872
31. Choi KS, Garyu J, Dumler JS 2003 Diminished adhesion of Anaplasma phagocytophilum-infected neutrophils to endothelial cells is associated with reduced expression of leukocyte surface selectin. *Infect Immun* 71:4586–4594
32. Sackstein R, Dimitroff CJ 2000 A hematopoietic cell L-selectin ligand that is distinct from PSGL-1 and displays N-glycan-dependent binding activity. *Blood* 96:2765–2774
33. Bevilacqua MP 1993 Endothelial-leukocyte adhesion molecules. *Annu Rev Immunol* 11:767–804
34. Johnston GI, Cook RG, McEver RP 1989 Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell* 56:1033–1044
35. Mengelers HJ, Maikoe T, Brinkman L, Hooibrink B, Lammers JW, Koenderman L 1994 Immunophenotyping of eosinophils recovered from blood and BAL of allergic asthmatic. *Am J Respir Crit Care Med* 149:345–351
36. Shimizu Y, Shaw S 1993 Cell adhesion. Mucine in the mainstream. *Nature* 366:630–631
37. Knol EF, Tackey F, Tedder TF, Klunk DA, Bickel CA, Sterbinsky SA, Bochner BS 1994 Comparison of human eosinophil and neutrophil adhesion to endothelial cells under nonstatic conditions. Role of L-selectin. *J Immunol* 153:2161–2167
38. Kogan TP, Dupre B, Bui H, McAbee KL, Kassir JM, Scott IL, Hu X, Vanderslice P, Beck PJ, Dixon RA 1998 Novel synthetic inhibitors of selectin-mediated cell adhesion: synthesis of 1,6-bis[3-(3-carboxymethylphenyl)-4-(2- α -D-mannopyranosyloxy)-phenyl]hexan (TBC1269). *J Med Chem* 41:1099–1111
39. Kogan TP, Dupre B, Bui H, McAbee KL, Kassir JM, Scott IL, Hu X, Vanderslice P, Beck PJ, Dixon RA 1998 Novel synthetic inhibitors of selectin-mediated cell adhesion: synthesis of 1,6-bis[3-(3-carboxymethylphenyl)-4-(2- α -D-mannopyranosyloxy)-phenyl]hexan (TBC1269). *J Med Chem* 41:1099–1111
40. Davenpeck KL, Berens KL, Dixon RA, Dupre B, Bochner BS 2000 Inhibition of adhesion of human neutrophils and eosinophils to P-selectin by the sialyl Lewis X antagonist TBC1269: preferential activity against neutrophil adhesion in vitro. *J Allergy Clin Immunol* 105:769–775