Translational inhibition of E-selectin expression stimulates P-selectin-dependent neutrophil recruitment

LENA OSTROVSKY, JULIANA CARVALHO-TAVARES, RICHARD C. WOODMAN, AND PAUL KUBES
Immunology Research Group and Departments of Physiology and Biophysics and Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Ostrovsky, Lena, Juliana Carvalho-Tavares, Richard C. Woodman, and Paul Kubes. Translational inhibition of E-selectin expression stimulates P-selectin-dependent neutrophil recruitment. Am J Physiol Heart Circ Physiol 278: H1225–H1232, 2000.—Although known for its role in hemostasis, there is a growing body of evidence that thrombin can induce leukocyte recruitment and contribute to the inflammatory response. An in vitro parallel-plate flow chamber was used to systematically examine thrombin-induced neutrophil interactions with human endothelium. Stimulation of endothelial cells with thrombin (1 U/ml) resulted in an immediate, P-selectin-dependent increase in neutrophil rolling and adhesion that was comparable in magnitude to optimal levels of histamine (the classical inducer of P-selectin). However, thrombin, but not histamine, induced a delayed (4 h) E-selectin-dependent rolling similar to that of tumor necrosis factor-α, suggesting that thrombin has the unique ability to recruit neutrophils by an early P-selectin and a delayed E-selectin pathway. Surprisingly, inhibition of E-selectin expression with the general protein synthesis inhibitor cycloheximide induced P-selectin expression 4 h after thrombin stimulation. Cycloheximide and thrombin (4 h) induced sufficient P-selectin-dependent rolling to recruit as many neutrophils as were recruited with 4 h of stimulation with thrombin alone. Histamine in the presence of cycloheximide or cycloheximide alone did not evoke the P-selectin response at 4 h, suggesting that this was not due to direct cycloheximide induction of P-selectin. Treatment of endothelium with tumor necrosis factor-α (an E-selectin inducer) and cycloheximide also eliminated E-selectin expression but, much like thrombin, induced P-selectin expression and neutrophil recruitment. In conclusion, inhibition of E-selectin via protein synthesis inhibition activates the protein synthesis-independent pathway of P-selectin expression to support adequate leukocyte recruitment.

leukocytes; endothelium; adhesion molecules; thrombin; tumor necrosis factor

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
serine protease to examine the interrelationship between P-selectin and E-selectin expression and/or function. In this study we used a systematic approach, including measurement of selectin expression as well as a parallel-plate flow chamber to obtain functional rolling data, and detailed the ability of thrombin to induce both endothelial selectin pathways of neutrophil recruitment in a human system under flow conditions. Our data reveal that thrombin induces rapid, P-selectin-dependent neutrophil rolling and adhesion over the first 30 min and delayed E-selectin-dependent leukocyte recruitment at 4 h. Surprisingly, inhibition of E-selectin protein synthesis inhibited the induction of the E-selectin pathway but invoked the protein synthesis-independent pathway of P-selectin expression that ensured adequate leukocyte recruitment. This was not the effect of protein synthesis inhibition per se, inasmuch as cycloheximide alone or in the presence of histamine (another P-selectin inducer) did not induce leukocyte recruitment at 4 h. This endothelial selectin switch from E-selectin to P-selectin was also observed for TNF, suggesting that the human system can overcome the inhibition of E-selectin with rapid mobilization of P-selectin and, for the first time, that there is intracellular cross talk for the two pathways.

MATERIALS AND METHODS

Antibodies. The blocking anti-P-selectin (G1) and the non-blocking anti-P-selectin (S12) antibodies were generously provided by Dr. R. P. McEver. The blocking anti-E-selectin antibody was purified from the supernatant of an antibody-secreting hybridoma (HB-10135, American Type Culture Collection, Rockville, MD). A second E-selectin antibody (EL-246) was used to measure E-selectin expression in ELISA (kindly provided by Dr. M. A. Jutila). The supernatant was first concentrated by centrifugation, and the antibody was purified using an IgG purification kit (Immunopure A, Pierce, Rockford, IL).

Cell culture. Human umbilical vein endothelial cells (HUVEC) were harvested from freshly obtained umbilical cords, as previously described (7, 12, 13). Briefly, umbilical cord veins were rinsed of formed blood products with warm PBS; then the vein was filled with collagenase (320 U/ml in PBS; Worthington Biochemical, Freehold, NJ). After 20 min of incubation in warm PBS, the cords were gently massaged to ensure detachment of endothelial cells from the vessel wall. The digest was collected into confluence tubes containing heat-inactivated fetal bovine serum to inactivate the collagenase and centrifuged (400 g for 10 min). The pellet was resuspended in medium 199 (GIBCO BRL, Grand Island, NY) supplemented with 20% fetal bovine serum and an antibiotic cocktail, but no endothelial cell mitogen. The cells were then seeded into fibronectin-coated T25 culture flasks and grown to confluence (2–5 days). On confluence, the HUVEC were rapidly detached with trypsin-EDTA (GIBCO BRL) and seeded heavily on fibronectin-coated glass coverslips. The heavy seeding and lack of mitogen in the media minimize cell growth and, thereby, permit cells to express P-selectin, which is otherwise lost (7). Consequently, only first-passage HUVEC were used for all experiments.

Neutrophil isolation. Human neutrophils were harvested from acetate-citrate-dextran-anticoagulated venous blood collected from healthy donors. All isolation steps were performed at room temperature. Neutrophils were purified by dextran sedimentation, then centrifuged through a Ficoll-Hypaque density gradient. Isolated neutrophils were resuspended in Hanks’ balanced salt solution buffer with Ca2+ and Mg2+ and used at a density of 106 cells/ml. The neutrophil suspensions were warmed in a 37°C water bath for 5 min before all flow chamber experiments.

Flow chamber assay. To study selectin-dependent neutrophil-endothelial cell interactions under shear conditions, a flow chamber assay was used as previously described (13). Glass coverslips with confluent monolayers of HUVEC were mounted into a polycarbonate chamber with parallel-plate geometry. The flow chamber was placed onto an inverted microscope stage, which was enclosed in a warm air cabinet, and the temperature was maintained at 37°C. A syringe pump (Harvard Apparatus) was used to draw the freshly isolated neutrophils over monolayers at a shear of 2 dyn/cm2. Neutrophil interactions with the endothelium were visualized and recorded using phase-contrast microscopy (×10 objective, ×10 eyepiece).

ELISA for cell surface adhesion molecule expression. Briefly, HUVEC were seeded at confluence into fibronectin-coated wells, treated with the appropriate stimuli, fixed, and blocked with 1% BSA. The endothelial cells were then labeled with 2 µg/µl of S12 (a nonblocking P-selectin monoclonal antibody (MAB)) or with 50 µg/ml of EL-246 (an E-selectin antibody) (15). Endothelial cells were then washed and labeled with a peroxidase-labeled goat anti-mouse IgG (1 µg/ml; Dako), washed a final time, and color developed with a tetramethyl benzidine one-step substrate system (Dako). The color reaction was stopped with 0.18 M H2SO4, and color was read on a plate reader at 450 nm.

Experimental protocol. The immediate (30 min) and prolonged (4 h) effects of thrombin on the endothelium were tested. To test the immediate effects of thrombin, neutrophils were perfused in buffer over unstimulated endothelial cell monolayers for a control period of 6 min. Then, at 6 min, thrombin (1 U/ml) was added to the neutrophil perfusion and was continually drawn over the HUVEC monolayers for the duration of the 30-min experiment. To test the prolonged effects of thrombin, HUVEC monolayers were kept in petri dishes, gently washed once with warm sterile PBS, stimulated with thrombin (1 U/ml) for 3 min, washed with PBS, and incubated in HUVEC media for 4 h. HUVEC monolayers were assembled into the flow chamber 4 h after stimulation, and then neutrophils were perfused across the monolayers. Preliminary experiments revealed that 1 U/ml of thrombin for 3 min elicited optimal neutrophil rolling and adhesion at 4 h, whereas higher concentrations of thrombin and/or longer exposure times of the endothelium to thrombin were cytotoxic. To ensure that endotoxin contamination was not mediating the thrombin-induced E-selectin effect, thrombin was mixed with polymyxin B cone before stimulation of endothelial cell monolayers. Polymyxin B was also present in HUVEC media for the 4-h period after HUVEC stimulation with thrombin. All flow chamber experiments were carried out for 30 min.

Protein synthesis was inhibited with cycloheximide (5–50 µg/ml) with and without thrombin, with histamine (a P-selectin but not an E-selectin inducer), and with TNF-α (an E-selectin but not a P-selectin inducer) and then examined at 4 h. This concentration of cycloheximide was used, inasmuch as it effectively inhibited TNF-α-induced E-selectin expression. For thrombin, 5 µg/ml of cycloheximide was sufficient to block E-selectin expression.

Statistics. All flow chamber data are reported as mean ± SE and run a minimum of three separate times on three separate cords. Most experiments were run at least eight times. Means were compared using the Mann-Whitney U test. Statistical significance was set at P < 0.05.
RESULTS

Thrombin stimulation of HUVEC rapidly induces neutrophil rolling. Figure 1 demonstrates that, under flow conditions, neutrophils perfused over unstimulated endothelial cell monolayers did not roll, whereas neutrophils began to roll within minutes of thrombin (1 U/ml) introduction into the perfusion buffer. Neutrophil rolling persisted for the duration of the experimental period. The rolling was completely P-selectin dependent, inasmuch as it was rapidly and completely inhibited by a blocking anti-P-selectin MAb (G1, 2 µg/ml) administered 20 min after thrombin stimulation of the endothelium (Fig. 1). If P-selectin antibody was not added, the number of rolling leukocytes did not decrease over the next 10 min (data not shown). We chose to stimulate the endothelium with 1 U/ml of thrombin, inasmuch as this concentration caused optimal neutrophil rolling, whereas higher concentrations caused endothelial injury. The induction of leukocyte rolling by thrombin was not due to the injurious effect of thrombin on the endothelial cell, inasmuch as addition of anti-thrombin III (an endogenous inhibitor of thrombin) to thrombin-treated endothelium, but not histamine-treated endothelium, reversed the rolling process (data not shown). Noteworthy was the very significant accumulation of cells on the thrombin-treated monolayer (Fig. 1B). In fact, most of the rolling neutrophils adhered over time rather than rolling through the field of view. Thrombin induced a rolling and adhesion profile similar to that of other rapid inducers of P-selectin, including histamine (Fig. 2). Neutrophil rolling velocity (15–20 µm/s) did not significantly differ

Fig. 1. Thrombin-induced leukocyte-endothelium interactions before and after thrombin stimulation. A: thrombin (1 U/ml) was added to perfusion buffer at 6 min, and neutrophils began to roll almost immediately. An anti-P-selectin antibody (G1, 2 µg/ml) was added at 28 min. B: effects of thrombin on neutrophil adhesion to endothelium.

Fig. 2. Histamine- and thrombin-induced rolling (A) and adhesive (B) interactions. Histamine (25 µM) or thrombin (1 U/ml) was added to perfusion buffer and perfused over endothelium. These concentrations of thrombin and histamine induced optimal responses in preliminary experiments. Leukocyte rolling and adhesion began minutes after stimulation with either agonist. *P < 0.05 relative to respective time-matched thrombin value.
between histamine and thrombin stimulation (data not shown).

HL-60 cells also roll on thrombin-stimulated endothelial cells. To ensure that the rolling was not a result of platelet contamination in neutrophil suspensions, we examined whether HL-60 cells, a myelomonocytic cell line, could also roll on thrombin-stimulated endothelium. As shown in Fig. 3, there were no HL-60-endothelial cell interactions on unstimulated endothelium during the control period. However, after thrombin stimulation, HL-60 cells began to roll within minutes, in a platelet-free system. The number of HL-60 cells rolling increased with time in a manner similar to that seen with neutrophils.

Prolonged endothelial stimulation with thrombin causes E-selectin-dependent neutrophil rolling. We next examined whether thrombin stimulation of endothelium would support neutrophil-endothelium interactions at 4 h. Figure 4A demonstrates a significant increase in neutrophil rolling 4 h after thrombin stimulation. The thrombin data in Fig. 4 are at the high end of the number of rolling cells observed at 4 h after thrombin stimulation, but generally the levels ranged between as few as 200 and as many as 1,600 cells per field of view. Figure 4B demonstrates that a significant number of neutrophils also adhered firmly to endothelium treated for 4 h. Because of the day-to-day variability, each experiment was completed with its own control.

When endothelial cell monolayers were stimulated with thrombin and polymyxin B (1 µg/ml; Sigma Chemical), a significant neutrophil recruitment was still noted, suggesting that the effects observed were due to thrombin and were not a result of endotoxin (data not shown).

Translational inhibition of thrombin-induced E-selectin upregulates P-selectin. Figure 5 is a summary of data for E-selectin and P-selectin expression during stimulation with thrombin or with thrombin and the protein synthesis inhibitor cycloheximide. Elevated levels of E-selectin expression but little P-selectin were noted on the endothelial surface 4 h after thrombin stimulation. The level of P-selectin was not different from baseline levels and failed to support rolling in flow chamber experiments. When protein synthesis was inhibited with cycloheximide, E-selectin expression was inhibited to baseline levels, whereas P-selectin expression was greatly increased. The adhesion molecule expression data paralleled the rolling results. At 4 h after thrombin stimulation, an anti-P-selectin MAb (G1) had

---

Fig. 3. HL-60 cells rolling on thrombin-stimulated endothelium. HL-60 cells were used at same concentration as neutrophils (10^6 cells/ml). Human umbilical vein endothelial cells (HUVEC) were stimulated with thrombin (1U/ml) at 6 min in a platelet-free system. HL-60 cells began to roll on endothelium minutes after thrombin stimulation, but not on unstimulated endothelium.

Fig. 4. Summary of leukocyte-endothelium interactions 4 h after thrombin stimulation. HUVEC monolayers were stimulated with thrombin (1 U/ml) for 3 min, gently washed with sterile PBS, and then incubated in HUVEC media for 4 h. Monolayers were assembled into flow chamber, and neutrophils were perfused across endothelial cells.
absolutely no effect on neutrophil rolling (Fig. 6). The anti-E-selectin antibody (7A9, 10 µg/ml) completely inhibited all neutrophil rolling at 4 h on endothelium treated with thrombin. When neutrophils were perfused over thrombin- and cycloheximide-treated endothelium, there was a 50% inhibition of neutrophil rolling, but there was still more than twice the amount of rolling as with acute thrombin treatment. However, in the presence of cycloheximide at a concentration that inhibited E-selectin synthesis (Fig. 5), anti-P-selectin antibody completely inhibited all rolling (Fig. 6). Cycloheximide on its own did not stimulate any rolling (Fig. 6).

Adhesion molecule switching does not occur with histamine. Figure 7 demonstrates that histamine (25 µM) caused a rapid (within minutes) increase in neutrophil rolling on endothelium. When neutrophils were perfused over endothelium 4 h after histamine stimulation, no interactions were observed, consistent with the view that the histamine-induced P-selectin effect is only short term. Moreover, because no neutrophil-endothelial interactions were noted, the data also suggest that histamine does not activate the E-selectin pathway. Under these conditions, when histamine was given with cycloheximide for 4 h, no rolling was noted at 4 h. These data support the view that cycloheximide does not impair the ability of P-selectin to be reinternalized from the surface of endothelium. Cycloheximide alone caused no leukocyte recruitment. Moreover, cycloheximide did not appear to have any detrimental effect on P-selectin expression, inasmuch as addition of histamine or thrombin 4 h after cycloheximide induced a normal rapid P-selectin response (data not shown).

Adhesion molecule switching occurs with TNF-α. To test the possibility that activation of the E-selectin synthesis pathway was necessary to induce rapid P-selectin expression in the presence of cycloheximide, we chose TNF-α, which induces E-selectin, but not P-selectin, expression. As shown in Fig. 8, control experiments, performed in the absence of cycloheximide, induced neutrophil rolling and adhesion on the endothelium that was inhibited by 7A9, an anti-E-selectin MAb. The remaining few cells rolled via a P-selectin-independent, unidentified mechanism. An L-selectin-dependent mechanism may be responsible for the residual leukocyte rolling. Neutrophils still rolled on HUVEC and adhered to HUVEC monolayers that were stimulated with TNF along with cycloheximide (Fig. 8). This rolling was...
completely inhibited by an anti-P-selectin antibody (Fig. 8).

DISCUSSION

Neutrophil recruitment to the site of injury is a stepwise process dependent on a cascade of events initiated by selectin-dependent rolling. The rolling interaction has been shown to be induced by various proinflammatory mediators and is critical for subsequent leukocyte adhesion and infiltration into tissues (9, 10, 19). Thrombin is a serine protease generated at sites of vascular injury and is primarily thought to play a key role in coagulation. It has received far less attention as an inflammatory mediator. However, there is a growing body of evidence that thrombin is also important in recruiting leukocytes in various inflammatory conditions, including ischemia-reperfusion and sepsis (4, 13). In this study we have demonstrated that thrombin induced immediate (minutes) and delayed (hours) neutrophil rolling and adhesion under flow conditions. The early neutrophil rolling was entirely mediated by P-selectin, whereas neutrophil rolling at 4 h was entirely mediated by E-selectin. There appeared to be no overlap between the two molecules, inasmuch as there was absolutely no E-selectin-dependent rolling at the early time point and no P-selectin-dependent rolling at 4 h after thrombin stimulation. The thrombin-induced E-selectin production was protein synthesis dependent, inasmuch as protein synthesis inhibition completely prevented E-selectin adhesion molecule expression and E-selectin-dependent neutrophil rolling. Surprisingly, when E-selectin upregulation was inhibited after thrombin stimulation, neutrophil recruitment was not prevented and was entirely mediated by P-selectin. This was not seen with another P-selectin inducer, histamine, but was observed with the E-selectin inducer TNF-α. To our knowledge, this is the first demonstration that translational inhibition of E-selectin leads to rapid P-selectin expression, suggesting potential cross talk between the endothelial selectins.

It is clear that, in humans, at least two endothelial selectin pathways for capturing neutrophils to the vessel surface have evolved, but the two pathways were thought not to overlap temporally to any significant degree. The first pathway is a rapid mobilization of presynthesized P-selectin from Weibel-Palade bodies that occurs within the first few minutes of endothelial stimulation but diminishes within 60–90 min. The second pathway is entirely dependent on protein synthesis and begins to express E-selectin on the surface of the endothelium at ~90–120 min. Therefore, as E-selectin expression is increased, P-selectin is reinternalized from the endothelial surface into endosomes and ultimately back into Weibel-Palade bodies. The exact reason for the evolution of two temporally distinct pathways remains unclear, inasmuch as both selectins recruit neutrophils (16). From a purely teleological view, the endothelium generates an early, temporary neutrophil recruitment mechanism before the body can synthesize sufficient protein to recruit leukocytes in a more prolonged fashion. Inasmuch as neutrophil recruitment from the circulation is the first line of defense against bacterial infiltration, it is conceivable that a very early and a delayed neutrophil recruitment system are required for optimal bacterial killing. However, our data demonstrate that the temporal relationship can be altered. Protein synthesis inhibition of the E-selectin pathway invokes the rapid mobilization of P-selectin at 4 h, which can recruit neutrophils in a very effective manner. The data demonstrate that the P-selectin pathway, in addition to being an early recruitment pathway, can also serve as a compensatory mechanism if the E-selectin pathway fails.

Another clear distinction between the P-selectin and the E-selectin pathway in humans is that very different mediators induce the two endothelial selectin pathways. P-selectin is expressed in response to histamine, cysteinyl leukotrienes, and oxidants, whereas E-selectin is synthesized in response to cytokines such as TNF-α and interleukin-1β. However, it would appear that thrombin could induce early P-selectin and later E-selectin expression (5, 8). In this study we extend these observations to demonstrate that both pathways are expressed in functional quantities in human systems, such that P-selectin and E-selectin can recruit neutrophils in response to thrombin under flow conditions. This permitted us to use thrombin as a tool to demonstrate that the two pathways are not entirely distinct from each other. Inhibition of E-selectin expression in thrombin-treated endothelium resulted in prolonged P-selectin expression well beyond the normal 60-min time frame. One potential explanation is cross talk between P-selectin and E-selectin in the human system. Evidence to support cross talk between P-selectin and E-selectin has been reported in

![Fig. 8. Stimulation of endothelium with tumor necrosis factor-α (TNF-α, 25 ng/ml) for 4 h without (left) or with (right) cycloheximide (50 µg/ml). Monolayers were assembled into flow chambers, and neutrophils were perfused across endothelial cells. In some experiments, an anti-P-selectin antibody or an anti-P-selectin and an anti-E-selectin antibody were tested. *P < 0.05 relative to TNF-α alone.](http://ajpheart.physiology.org/)

**H1230 THROMBIN-INDUCED LEUKOCYTE RECRUITMENT**
mice (6). Antigen-induced leukocyte recruitment at 4 h in wild-type mice was entirely dependent on P-selectin on the basis of antibody inhibition studies. However, when the same experiment was performed in P-selectin-deficient mice, E-selectin was produced in sufficient quantities to recruit adequate numbers of leukocytes. Clearly, the system compensates in the P-selectin knock-out mice, such that deletion of one endothelial selectin may activate the expression of the other endothelial selectin.

Although the data do support the view that cross talk exists between the two endothelial selectin pathways in the human system, there may be a number of alternative explanations unrelated to thrombin. First, protein synthesis inhibition is known to activate the stress kinase pathway (21), which may cause the ongoing P-selectin expression. However, this is unlikely, inasmuch as cycloheximide alone did not induce P-selectin expression at 4 h. Alternatively, cycloheximide blocks all protein synthesis, and so it is possible that, in addition to inhibiting E-selectin synthesis, this compound may inhibit synthesis of proteins required for the final internalization and processing of P-selectin. However, this is also an unlikely possibility, inasmuch as exposure of endothelium to cycloheximide and histamine for 4 h did not cause P-selectin expression at 4 h, despite the fact that histamine was as effective as thrombin at inducing early P-selectin expression. Clearly, activation of the endothelium or stimulation with histamine was not sufficient to induce delayed P-selectin expression after protein synthesis inhibition.

An alternative explanation is that translational inhibition of E-selectin was sufficient to activate the P-selectin pathway. To test this possibility, we used a mediator (TNF-α) that activated E-selectin, not P-selectin. Indeed, when TNF-α was used to stimulate the endothelium in the presence of protein synthesis inhibition, neutrophil recruitment was still visible at 4 h and was almost entirely mediated by P-selectin. These data demonstrate for the first time that inhibition of E-selectin induces the P-selectin pathway to allow for neutrophil recruitment. The data also suggest that this observation is not particular for thrombin, which has the distinctive characteristic of stimulating P-selectin and E-selectin, but may be a general phenomenon for any E-selectin inducer.

The reduction in P-selectin expression with time is not due to depletion of P-selectin stores, inasmuch as within 10–20 min of expression of P-selectin to the surface of endothelium, P-selectin is reinternalized into endosomes and then to the Golgi region and, finally, is recycled into Weibel-Palade bodies for further reexpression (18). Therefore, a mechanism must be in place that interrupts the reexpression of P-selectin. One possibility is that the induction of E-selectin automatically signals a turn-off mechanism for the P-selectin pathway. Our data suggest that if E-selectin expression is inhibited, then P-selectin expression remains elevated on endothelium; however, the converse is unlikely to be true. For example, P-selectin is downregulated after histamine stimulation, despite the fact that histamine does not induce E-selectin. Moreover, we have induced P-selectin expression with TNF-α and at 4 h still obtain P-selectin with histamine (unpublished observations), suggesting that E-selectin can be produced and yet rapid mobilization of P-selectin can still occur. Clearly, induction of E-selectin does not automatically translate into inhibition of P-selectin production.

In conclusion, our data reveal potential redundancy among the selectins in human endothelium. Although the redundancy was only apparent after protein synthesis inhibition, this is not trivial, inasmuch as many of the anti-inflammatory drugs (e.g., glucocorticoids, cyclosporin A), as well as newly developed therapeutic agents specifically designed to target transcription factors, impact on protein synthesis and, therefore, could conceivably still induce P-selectin expression. With this in mind, optimal inhibition of leukocyte recruitment may not be achieved by these and other anti-inflammatory drugs and may require targeting of both endothelial selectins for complete inhibition of leukocytes.

The authors thank Nursing Unit 51 of the Foothills Hospital for providing the umbilical cords from which endothelial cells were obtained.

This study was supported by a grant from the Bayer Inc. of Canada/Canadian Red Cross Society Research and Development Fund. J. Carvalho-Tavares is supported by a Heart and Stroke Foundation Fellowship. P. Kubes is a Medical Research Council Scientist. P. Kubes and R. C. Woodman are scholars of the Alberta Heritage Foundation for Medical Research.

Address for reprint requests and other correspondence: P. Kubes, Immunology Research Group and Dept. of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, AB, Canada T2N 4N1.

Received 26 July 1999; accepted in final form 28 October 1999.

REFERENCES


