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7. Hato Masaguaral (67°35′W, 8°34′N), is located along a tributary of the Guárico River, in the central plains (llanos) of Guárico state, Venezuela.
8. Of a total of 51,704 bird-minutes of observation at the study site, parts of plants eaten by hoatzins were: buds, shoots, and new leaves, 67.8%; mature leaves, 12.6%; flowers, 10.4%; fruits, 8.1%; and unidentified leaves, 1.1%. Green leaves of *Zanthoxylum culantrillo* (Rutaceae), *Acacia articulata* (Fabaceae), *Lonchocarpus cruciarubieres* (Fabaceae), and *Guazuma ulmifolia* (Sterculiaceae) make up 56% of what was eaten in the observed foraging time during the breeding season.
9. Eaten portions are significantly higher in water content and crude protein ($P < 0.001$) and lower in total cell wall, lignin, cellulose, and hemicellulose ($P < 0.01$) than uneaten portions (two-tailed Mann-Whitney U test). No significant differences were seen for cutin and soluble phenolics. Average nutritional value of the natural diet is (in percentage dry matter) 73% water content, 78% organic matter (OM), 46% neutral detergent fiber (NDF), 32% acid detergent fiber (ADF), 17% cellulose, 13% hemicellulose, 10% lignin, and 18% total nitrogen (N) [nutritional analysis followed H. K. Goering and P. J. VanSoest, *Agriculture Handbook 379* (U.S. Department of Agriculture, Washington, DC, 1970)]. Soluble phenolics were measured by acetone extraction.
10. Quantitative descriptions of the digestive morphology and foregut fermentation of hoatzins were based on ten individuals collected between 1983 and 1988. The *p*H of gut contents was measured at the time of collection. Digesta from potential fermentation sites was preserved in sulfuric acid solution. Concentrations of volatile fatty acids (VFA) were measured by gas chromatography [A. Wilkie, M. Goto, F. M. Bordeaux, P. H. Smith, *Biomass* **11**, 135 (1986)]. Segments of the gut were isolated and weighed to examine relative capacity when full and emptied of their contents. Digesta from all segments of the gut was collected for particle size analysis and comparative nutritional analysis.
11. Microflora was mostly composed of gram-negative rods with an average concentration of 1.10×10^9 per milliliter ($n = 6$, SD = 0.03×10^9). No protozoans were found.
12. Mean particle size was measured with a computerized particle analysis video system with a camera mounted on a microscope. Mean particle size was significantly different between the upper esophagus and the lower esophagus before the entrance of the proventriculus [290 μm (SD = 119) and 158 μm (SD = 36), respectively]. Two-tailed Mann-Whitney U test ($P = 0.03$, $n = 5$).
13. Mean retention times were calculated as $t = \Sigma m_i t_i / \Sigma m_i$, where m_i is the amount of marker excreted per unit dry matter at the ith defecation at time t after dosing ($n = 3$, SD = 2.9, 7.2, and 5.7). We used commercial flagging tape as the 1- and 8-mm^2 markers and plastic beads as the 3-mm^3 markers. All markers had a specific gravity of 1.01. Particles are selectively retained in the narrow passages between the anterior and posterior chambers of the crop and the extensive sacculation of the caudal esophagus.
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15. Offered and excreted fractions were quantitatively recovered and expressed in percentage of dry matter (%DM at 105°C). The nutritional composition of an experimental diet of romaine lettuce, ground alfalfa pellets, and soybean protein concentrate was 20% DM, 19% NDF, 18% ADF, 11% cellulose, 25% N, and 81% OM. Captive hoatzins ($n = 4$) maintained a constant body mass during and after the experiment. Average intake rate was 44.3 g(DM)/day (SD = 8.07). Apparent digestibilities for dietary fractions ([1 − (DM excreted/DM ingested)] × 100) were: 77.1 ADF, 59.5 cellulose, and 71.4 OM. In vitro OM digestibility of the experimental diet by cow ruminal inoculum was 77.6. Apparent digestibilities may be underestimated, since no separation of urinary and fecal products was possible.
16. Samples of 100 mg of dried ground young alfalfa (39.5% cell wall) were incubated with 10 ml of inoculum for 3, 6, 12, 24, 48, and 72 hours in 20 ml screw-cap Hungate tubes under anaerobic and isothermic conditions. The inoculum consisted of a 1:5 (w:w) dilution of hoatzin crop content (or cow rumen content) in a standard buffering solution (McDougall's artificial saliva). Rates of in vitro cell wall digestibility were the slope of regression curves of the natural logarithm (ln) transformation of percentage digestibilities on hours of fermentation. For cow rumen $y = 0.0096x + 3.647$ ($n = 13$, slope SE = 0.0035) and for hoatzin crop extract $y = 0.0076x + 3.94$ ($n = 12$, slope SE = 0.0011). In vitro cell wall digestibilities after a 72-hour incubation were 80.2 and 79.9 (SD = 19.32 and 8.37; $n = 5$ and 3) for cow and hoatzin, respectively.
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20. We dedicate this work to the memory of R. Parra, who passed away in February 1988. His ideas and enthusiasm will always be with us. Funding was provided by the New York Zoological Society, Universidad Central de Venezuela, Consejo Nacional de Ciencia y Tecnología de Venezuela (CONICIT), Organization of American States (OAS), and the Smithsonian Institution. S.D.S. thanks E. Dolensek for his guidance in early phases of the study. We thank O. Parra and the personnel of the Laboratory of Forage Analyses at UCV Maracay, T. Blohm for permitting our work at Masaguaral, and many field assistants. K. A. Bjorndal, D. J. Levey, C. Martinez del Rio, and B. K. McNab made valuable editorial comments.

19 May 1989; accepted 14 August 1989

Neutrophil Mac-1 and MEL-14 Adhesion Proteins Inversely Regulated by Chemotactic Factors

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The neutrophil Mac-1 and gp100^{MEL-14} adhesion proteins are involved in neutrophil extravasation during inflammation. Both the expression and activity of Mac-1 are greatly increased after neutrophil activation. In contrast, neutrophils shed gp100^{MEL-14} from the cell surface within 4 minutes after activation with chemotactic factors or phorbol esters, releasing a 96-kilodalton fragment of the antigen into the supernatant. Immunohistology showed that gp100^{MEL-14} was downregulated on neutrophils that had extravasated into inflamed tissue. The gp100^{MEL-14} adhesion protein may participate in the binding of unactivated neutrophils to the endothelium; rapid shedding of gp100^{MEL-14} may prevent extravasation into and damage of normal tissues by activated neutrophils.

CIRCULATING AND BONE MARROW neutrophils provide a front line of defense that can be rapidly mobilized and activated against infectious agents. The first step in extravasation involves adhesive interactions of neutrophils with the vascular endothelium, which must be regulated to allow localization of neutrophils only to inflammatory sites. Similarly, activation of neutrophils must be limited to those cells at the inflammatory site, so that damage to normal tissues is minimized. The leukocyte integrins, LFA-1, Mac-1, and p150,95 [collectively referred to as the CD18 complex (*1*)], are essential in the neutrophil extravasation process, as demonstrated experimentally (*2, 3*) and from the study of humans with a genetic deficiency of CD18 expression (*4*). Optimal CD18 function requires cell activation (*4–7*), which is also accompanied by rapid mobilization of an intracellular pool of Mac-1 and p150,95 to the cell surface (*8*). A second class of neutrophil adhesion protein, gp100^{MEL-14}, has been implicated in neutrophil extravasation (*9, 10*). Neutrophil gp100^{MEL-14} is related to lymphocyte gp90^{MEL-14}, which mediates adhesion to the specialized high endothelial venules (HEV) of peripheral lymph nodes (*11*). The MEL-14 monoclonal antibody (MAb) prevents murine neu-

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trophil localization to subcutaneous inflammatory sites (*9*) and to the inflamed peritoneum (*9, 10*) in vivo, and blocks binding of neutrophils to vascular endothelium within tissue sections of lymph nodes in vitro (*9, 10*). In contrast to Mac-1 activity, gp100^{MEL-14}–mediated binding does not appear to require neutrophil activation. Indeed, phorbol myristate acetate (PMA)–activated neutrophils or neutrophils harvested from inflamed peritoneum have increased expression of Mac-1 but, in direct contrast, a decrease to one-tenth the gp100^{MEL-14} expression of circulating neutrophils (*10*).

We examined the effects of a panel of physiologic mediators of neutrophil activation on in vitro gp100^{MEL-14} expression (Fig. 1). Murine bone marrow and peripheral blood neutrophils have similar concentrations of gp100^{MEL-14} and show similar ability to localize to inflammatory sites in vivo (*9, 10*). Because of the inaccessibility of blood neutrophils in the mouse, we chose to study bone marrow neutrophils. Neutrophils were activated with various levels of mediators for 1 hour at 37°C. Neutrophil chemotactic factors, such as the C5a fragment of complement and leukotriene B_4 (LTB_4), were potent inducers of gp100^{MEL-14} downregulation at concentrations of $1 \times 10^{-8}M$ (Fig. 1, D and F). Downregulation with PMA (100 ng/ml) was more complete (Fig. 1C), perhaps reflecting heterogeneity among the bone marrow neutrophils for expression of C5a and LTB_4 receptors. Tumor necrosis factor (TNF), which has chemotactic activity (*12*), was also effective at concentrations of 10 to 500 U/ml (Fig. 1E). Interleukin-1 (IL-1) (Fig. 1H) and lipopolysaccharide (LPS) (Fig. 1G) reduced gp100^{MEL-14} expression, but only at relatively high doses (200 U/ml and 1 to 10 μg/ml, respectively). All of these factors also induced Mac-1 upregulation (Fig. 1, I to P). In general, the extent of Mac-1 increase correlated with the extent of gp100^{MEL-14} decrease. Incubation for 1 hour at 37°C in the absence of any mediators (Fig. 1J) also resulted in partial Mac-1 upregulation, as previously described (*5*), and partial gp100^{MEL-14} downregulation (Fig. 1B). Other factors, such as concanavalin A, interferon-γ, and transforming growth factor-β did not reduce gp100^{MEL-14} expression more than the 37°C control (*13*). In separate experiments, gp100^{MEL-14} was also downregulated on peripheral blood neutrophils upon activation with PMA and C5a (*13*).

Next we studied the kinetics of gp100^{MEL-14} downregulation on activated neutrophils (Fig. 2). Murine bone marrow neutrophils were activated with C5a ($1 \times 10^{-8}M$) for various lengths of time, rapidly fixed by the addition of paraformaldehyde (0.5% final concentration), and then analyzed by flow cytofluorometry. Downregulation of gp100^{MEL-14} expression was rapid, with a 60% reduction detectable 1 min after exposure to C5a (Fig. 2, compare A and B).

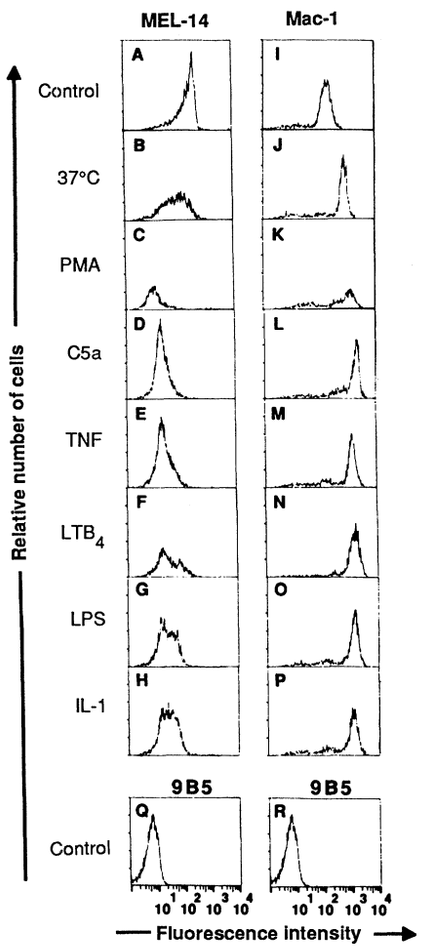

Fig. 1. Chemotactic factors are potent regulators of gp100^{MEL-14} expression. Bone marrow leukocytes (BML) were isolated from femurs of BALB/c mice (*9, 10*). The gp100^{MEL-14}–positive cells are 80% neutrophils and are competent for localization to inflammatory sites in vivo (*9, 10*). The BML were untreated (**A** and **I**), or incubated at 37°C for 1 hour in the absence of stimulus (**B** and **J**) or in the presence of PMA (100 ng/ml) (**C** and **K**), C5a ($10^{-8}M$) (**D** and **L**), TNF (100 U/ml) (**E** and **M**), LTB_4 ($10^{-8}M$) (**F** and **N**), LPS (10 μg/ml) (**G** and **O**), or Il-1 (1 μg/ml) (**H** and **P**) and then stained with MAb against gp 100^{MEL-14} [MEL-14 (*11*), panels A to H] or Mac-1 [M1/70 (*26*)] (I to P), or with a control MAb [9B5 (*27*)] (**Q** and **R**) by indirect immunofluorescence. The cells were analyzed by quantitative flow cytometry (FACStar, Becton-Dickinson). The neutrophil population was gated by characteristic forward- and right-angle light-scatter profiles (*9, 10*).

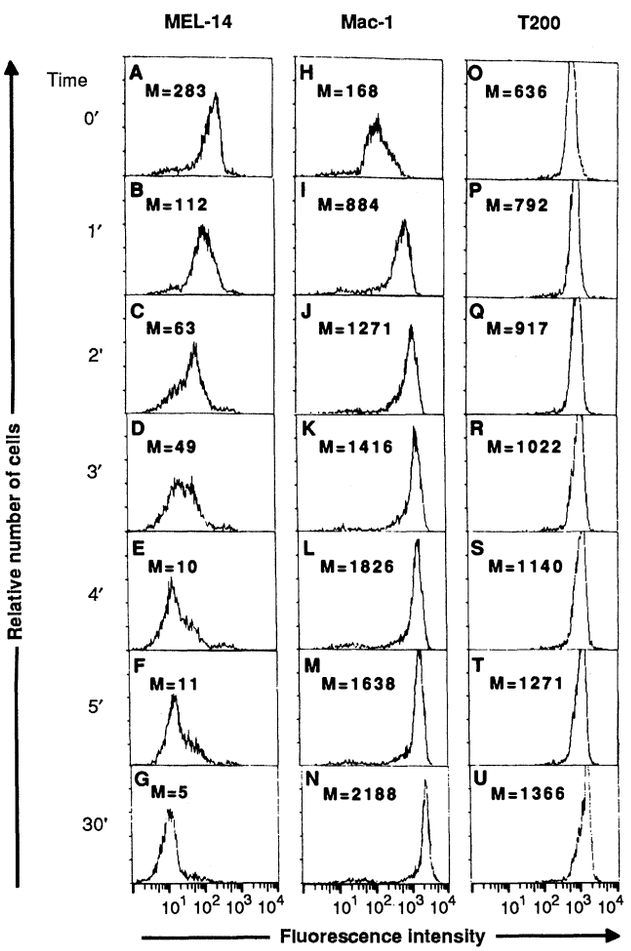

Fig. 2. Kinetics of gp 100^{MEL-14} and Mac-1 regulation by C5a. Murine bone marrow neutrophils were activated at 2×10^7 cells per milliliter with $1 \times 10^{-8}M$ recombinant human C5a (Sigma) for various lengths of time at 37°C. The neutrophils were then rapidly fixed by the addition of an equal volume of 1% paraformaldehyde in phosphate-buffered saline (PBS). The fixed cells were washed, then stained with MAb against gp100^{MEL-14} (**A** to **G**), Mac-1 (**H** to **N**), T200 [30G12 MAb (*28*)] (**O** to **U**), or with a control MAb (not shown) by indirect immunofluorescence and analyzed by flow cytometry. Mode linear fluorescence values (M) of each sample minus mode linear fluorescence values of control samples are presented with each histogram. Mode linear fluorescence values of control samples at time 0, 1, 2, 3, 4, 5, and 30 min were 7, 6, 5, 6, 5, 5, and 6, respectively.

Downregulation of gp100^{MEL-14} continued steadily and was almost complete by 4 min (Fig. 2, B to E), although gp100^{MEL-14} expression continued to decrease slightly at 30 min (Fig. 2G). The gp100^{MEL-14} was almost completely lost 5 min after activation with PMA, and a 5-min incubation with LTB_4 reduced staining by 78% (*13*). This rapid modulation of gp100^{MEL-14} closely followed the known kinetics for upregulation of Mac-1 [(*4*) and Fig. 2, H to N]. In contrast, expression of the T200 cell surface protein only increased slightly (Fig. 2, O to U), which is consistent with reports of mobilization of a small intracellular pool of T200 (*14*). At short time points (5 and 10 min), LPS and IL-1 had no effect on gp100^{MEL-14} expression, suggesting that these factors may cause gp100^{MEL-14} downregulation indirectly, perhaps by inducing endogenous LTB_4 production (*15*). These results indicate that chemotactic factors are potent inducers of gp100^{MEL-14} downregulation.

The rapid downregulation of gp100^{MEL-14} could be due to internalization of gp100^{MEL-14}, shedding of gp100^{MEL-14}, or a conformational change that results in the loss of the MEL-14 epitope. To distinguish between these possibilities, we used a polyclonal MEL-14 serum to immunoprecipitate gp100^{MEL-14} from both the cell lysate and the cell-free supernatant of neutrophils surface-labeled with ^{125}I and activated with PMA. No MEL-14 antigen could be detected in association with the cell lysates of activated neutrophils (Fig. 3, lane 1). In contrast, MEL-14 antigen was immunoprecipitated from the supernatant of these activated cells (Fig. 3, lane 4). The shed MEL-14 antigen was only about 4 kD smaller than gp100^{MEL-14} associated with unactivated neutrophils (Fig. 3, compare lanes 4 and 7), a size that is consistent with the large extracellular domain predicted by MEL-14 cDNA clones (*16*). A small amount of MEL-14 antigen is detectable in the supernatant of control cells (Fig. 3, lane 10), which may represent a normal low-level release of MEL-14 antigen or partial activation of neutrophils during the isolation and iodination procedures. The T200 antigen was not detectable in supernatant from either activated or unactivated cells (Fig. 3, lanes 5 and 11).

Neutrophil interaction with venules in vivo and subsequent diapedesis is thought to occur in 3 to 10 min, a time frame consistent with the in vitro changes in gp100^{MEL-14}. To determine whether rapid gp100^{MEL-14} downregulation is associated with extravasation in vivo, we examined inflamed tissues from mice by immunohistology. The footpads of mice were inflamed with 3 ng LPS for 3 hours, and then the mice were infused with the dye luconyl blue (*17*) to stain vessels (Fig. 4A). Frozen thin sections of the inflamed footpads were prepared and stained by two-color analysis with antibody to Mac-1 (anti-Mac-1) and MEL-14 MAb. Many neutrophils within the lumen or walls of small vessels associated with inflamed tissues were both Mac-1$^+$ and MEL-14$^+$ (*13*). In contrast, all neutrophils that had extravasated into the surrounding inflamed tissue stained intensely with anti-Mac-1 (Fig. 4B), but not with MEL-14 MAb (Fig. 4C). Interestingly, in some larger vessels, neutrophils within the lumen were also MEL-14$^-$ (Fig. 4C). These latter cells were often associated in small aggregates (Fig. 4B), a phenomenon that is known to be activation dependent in vitro (*4, 5*). Since unstimulated peripheral blood

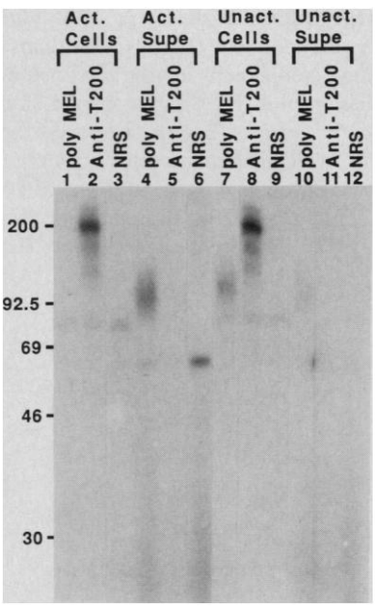

Fig. 3. The gp100^{MEL-14} is shed from the cell surface of activated neutrophils. We washed the neutrophils three times in PBS and surface-labeled them with ^{125}I using lactoperoxidase beads (Bio-Rad) according to manufacturer's recommendations. Labeled neutrophils were incubated in RPMI containing 1% bovine serum albumin (BSA) for 10 min at 37°C with (lanes 1 to 6) or without (lanes 7 to 12) PMA (100 ng/ml). Proteins were immunoprecipitated from lysates of washed cells (lanes 1 to 3 and 7 to 9) or from 12,000*g* supernatants of the cells (lanes 4 to 6 and 10 to 12). The gp100^{MEL-14} (lanes 1, 4, 7, and 10) was immunoprecipitated with a polyclonal rabbit antiserum to MEL-14 (poly MEL) and protein A–coupled agarose beads. The T200 antigen was immunoprecipitated with the 30G12 MAb (anti-T200) (*28*) and antibody to rat immunoglobulin G coupled to agarose beads (lanes 2, 5, 8, and 11). Control precipitations were done with normal rabbit serum (NRS) and protein A–coupled agarose beads (lanes 3, 6, 9, and 12). The immunoprecipitates were subjected to SDS–8% polyacrylamide gel electrophoresis under reducing conditions and autoradiography. Migration of molecular mass standards is indicated. Act., activated; Supe, supernatant; Unact., unactivated.

neutrophils are MEL-14$^+$ (*9, 10*), the demonstration of MEL-14$^-$ neutrophils within vessels of inflamed tissues indicates that gp100^{MEL-14} downregulation can occur early during extravasation and is rapid in vivo as well as in vitro. The immunohistologic demonstration of MEL-14$^-$ neutrophils surrounding inflamed venules provides direct evidence for neutrophil activation during the extravasation process.

The inverse effects of activation on expression of Mac-1 and gp100^{MEL-14} demonstrated here suggest that these two proteins mediate distinct but complementary events. The rapid downregulation of gp100^{MEL-14} in association with extravasation in vivo, and in conjunction with activation in vitro, indicates that its participation must be confined to early stages of extravasation, preceding neutrophil activation in inflammatory sites. Neutrophil activation does not appear to be required for gp100^{MEL-14}–mediated adherence, as evidenced by rapid, MEL-14–inhibitable binding of unactivated neutrophils to endothelium within frozen sections of tissue at 7°C (*9, 10*). It is likely that gp100^{MEL-14} is an adhesion molecule, probably acting through its lectin domain (*16*) with carbohydrate determinants induced on endothelial cells (*18*). The shedding of gp100^{MEL-14} could provide a rapid "de-adhesion" mechanism and may be necessary for the neutrophil to proceed from binding to the endothelium to migrating through the endothelium or for release of the neutrophil from the endothelium after diapedesis. Alternatively, gp100^{MEL-14} downregulation could act as a protective mechanism that prevents activated neutrophils, freed by shear forces from inflamed venules, from entering secondary sites which are unrelated to the initial insult. In support of this model, when activated (Mac-1^{bright}/MEL-14^{dull}) neutrophils are injected intravenously they are unable to localize to inflamed tissues in vivo (*10*).

In contrast, numerous reports have underscored the importance of neutrophil activation for both increased Mac-1 expression and functional activity. Indeed, studies (*19, 20*) suggest that CD18 does not contribute significantly to the interaction of unstimulated neutrophils to endothelial cells under conditions of physiologic shear force, but instead may be important in cementing initial adhesive events mediated by other mechanisms and in transendothelial migration (and in subsequent neutrophil effector functions). This model is consistent with the observation that antibody to CD18 inhibits irreversible attachment of neutrophils to inflamed venules in vivo, but does not affect initial neutrophil-endothelial cell interaction and rolling (*2*). These reports add to the

increasing evidence for a Mac-1–independent mechanism of adhesion of resting neutrophils to cultured endothelial cells (*7, 19–22*). Other CD18 adhesion proteins, LFA-1 and p150,95, may account for some of this Mac-1–independent adhesion (*7, 21*). However, unstimulated neutrophils from CD18-deficient patients are still competent for binding to cultured endothelial cells (*7, 20, 23*). Indeed, activation of CD18-deficient neutrophils actually reduces adhesion of these cells to endothelial cells (*24*), suggesting that some CD18-independent adhesion function may be downregulated on activation. We developed MAbs against the human homolog of gp100^{MEL-14} in order to examine the role of this antigen in such CD18-independent adhesion in vitro (*25*).

In conclusion, we hypothesize that gp100^{MEL-14}, perhaps in conjunction with yet unidentified adhesion systems, is involved in mediating the initial adhesion event between circulating neutrophils and the vascular endothelium adjacent to inflammatory sites. Chemotactic factors released at the inflamed site may initiate the activation process in these adherent cells, downregulating gp100^{MEL-14} and engaging Mac-1 activity to allow extravasation to occur. Neutrophil interactions with venules at sites of inflammation thus reflect a complex interplay between cell adhesion-recognition molecules and cellular activation events.

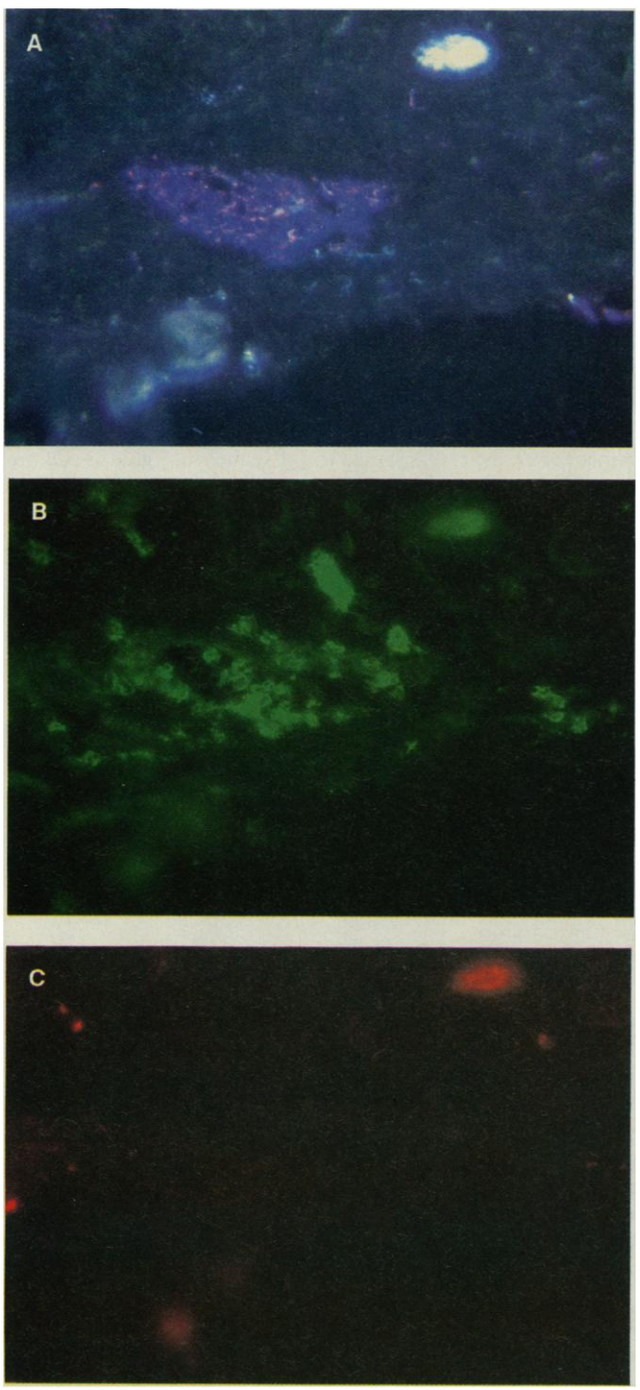

Fig. 4. Downregulation of gp100^{MEL-14} occurs rapidly in vivo during inflammation. Thin sections of footpads of mice which were inflamed after a 3-hour exposure to LPS, were first stained with MEL-14 then with PE-conjugated goat antibody to rat immunoglobulin G (**C**), blocked with rat serum, and then stained with fluorescein isothiocyanate (FITC)–conjugated MAb to Mac-1 (**B**). The identification of vessels was aided by infusing mice intravenously with the dye luconyl blue just before they were killed (**A**). Many areas of the inflamed tissue contained MEL-14$^+$ cells localized within vessels (*13*). However, there were no obvious examples of MEL-14$^+$ cells that were not associated with a vessel. The neutrophil-specific MAb RB6-8C5 (*9*) gave a staining pattern similar to that with MAb to Mac-1, suggesting that most of the Mac-1$^+$ cells observed in these inflamed tissues were neutrophils (*13*).

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29. We thank L. Rott for assistance with flow cytofluorometry; S. Rosen for the polyclonal serum against MEL-14 antigen; C. W. Smith for sharing data and for discussions; and R. Bargatze, L. Picker, and R. Hallman for critical review of the manuscript. Supported by NIH grant AI 19957; an Established Investigator Award of the American Heart Association (E.C.B.); an American Cancer Society, CA Division Fellowship (M.A.J.); and a Damon Runyon–Walter Winchell Cancer Foundation Fellowship (T.K.K.).

2 June 1989; accepted 1 August 1989