Identification of a human peripheral lymph node homing receptor: A rapidly down-regulated adhesion molecule

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ABSTRACT Lymphocyte migration to lymphoid organs involves organ-specific homing receptors. The mouse peripheral lymph node homing receptor, defined by the MEL-14 monoclonal antibody (mAb), is a lectin-like cell surface protein, which is rapidly down-regulated upon cell activation with phorbol 12-myristate 13-acetate. We have raised mAbs against rapidly shed molecules released from the cell surface of activated human leukocytes. Five mAbs, DREG-55, -56, -110, -152, and -200, define an 80- to 85-kDa molecule involved in human lymphocyte recognition of peripheral lymph node (PLN) high endothelial venules (HEVs). The DREG-56 mAb specifically inhibits >90% of binding of human lymphocytes to HEVs within frozen sections of peripheral but not mucosal lymphoid tissue. Furthermore, the gp80 antigen is expressed on lymphoid cell lines that are capable of binding to PLN HEVs. The DREG-56 mAb also inhibits lymphocyte binding of the phosphomannan monoester core from *Hansenula hostii* Y-2448, an activity associated with human and mouse lymphocyte recognition of PLN HEVs. Finally, all five DREG mAbs specifically stain COS cells transfected with LAM-1 cDNA, a putative human homologue of mouse MEL-14 antigen. These results demonstrate that the DREG mAbs define a human lymphocyte homing receptor for PLN HEVs and indicate that this human antigen is homologous to the MEL-14-defined murine lymphocyte homing receptor.

Lymphocytes, monocytes, and neutrophils are by necessity mobile cells that require access to distant tissues for maintaining normal distribution of immune cells throughout the body and for mounting inflammatory responses in localized sites of infection. Lymphocytes in particular are long-lived cells that continuously recirculate between specialized lymphoid organs via the bloodstream and lymph. A salient feature of recirculation is the ability of lymphocytes to leave the bloodstream via specialized endothelial cells, which comprise the morphologically distinct, postcapillary high endothelial venules (HEVs) found in lymphoid tissues and sites of chronic inflammation (ref. 1; reviewed in ref. 2). Lymphocytes bind with exquisite specificity to HEVs within frozen sections of lymphoid tissues *in vitro* (3, 4). Lymphocyte binding to peripheral lymph node (PLN), gut-associated (mucosal), and synovial (chronically inflamed) HEVs utilize distinct recognition mechanisms (2, 5–9).

The mouse MEL-14 antigen, the first homing receptor characterized, fulfills the criteria expected of an organ-specific lymphocyte homing receptor (5). Expression of the MEL-14 antigen on lymphocytes is developmentally regulated and correlates with HEV binding ability *in vitro* and migratory behavior *in vivo* (2, 5). The MEL-14 monoclonal antibody (mAb) blocks lymphocyte binding to HEVs in PLNs, but not in Peyer's patches, and specifically inhibits lymphocyte homing to PLNs *in vivo* (5). It is possible that other adhesion molecules work in concert with the MEL-14

antigen to determine organ specificity. The MEL-14 antigen appears to be a mammalian lectin (10–14), probably interacting with unique carbohydrate determinants on peripheral node HEVs. The MEL-14 antigen is also involved in neutrophil (15, 16) and monocyte (ref. 17; M.A.J. and E.C.B., unpublished data) extravasation during acute inflammation. Thus, the MEL-14 antigen participates in a variety of leukocyte–endothelial cell interactions.

A human PLN homing receptor analogous in function and expression to the mouse MEL-14 antigen has not been described. Clearly, the identification of a human peripheral node-specific homing receptor is an important goal, both for understanding the physiology of human lymphocyte traffic and for its potential clinical diagnostic and therapeutic value.

We have recently found that the mouse MEL-14 antigen is shed from the surface of neutrophils within minutes after activation with phorbol 12-myristate 13-acetate (PMA) or chemotactic factors (18). A large fragment of the MEL-14 antigen, corresponding in size to the predicted extracellular domain, can be quantitatively recovered from the supernatant of activated neutrophils. Furthermore, MEL-14 antigen on PMA-activated lymphocytes (refs. 19 and 20; M.A.J., T.K.K., and E.C.B., unpublished observations) and monocytes (unpublished observations) appears to be down-regulated in a similar manner, although with different kinetics (20). We have used this observation to devise a strategy to raise mAbs against similarly regulated molecules on human cells that may be involved in lymphocyte homing. Here we describe a down-regulated adhesion molecule (gp80), which has lectin-like binding activity and specifically mediates human lymphocyte binding to PLN HEVs.

METHODS

Cells. Human peripheral blood leukocytes were isolated by dextran T500 sedimentation. In some experiments, peripheral blood leukocytes and peripheral blood mononuclear cells were isolated by a single step Ficoll/Hypaque centrifugation (Histopaque-1119 and -1077, respectively; Sigma).

Human B-lymphoblastoid and lymphoma cell lines were kindly provided by the late H. Kaplan (LB25, LBF) (Stanford University), E. Engleman (IBW4, KCA) (Stanford University), L. Herzenberg (MOLT-4) (Stanford University), and S. Smith (KW) (Stanford University). These cell lines have been previously shown to bind to PLN HEVs (LB25), Peyer's patch HEVs (KCA), both PLN and Peyer's patch HEVs (LBF and IBW4), or to be HEV nonbinders (KW, CA46, and MOLT 4) (6).

mAbs. Human peripheral blood leukocytes ($3\text{--}5 \times 10^8$; 5×10^7 cells per ml in RPMI 1640 medium containing 0.1%

Abbreviations: PLN, peripheral lymph node; HEV, high endothelial venule; PMA, phorbol 12-myristate 13-acetate; FITC, fluorescein isothiocyanate; PPME, phosphomannan monoester core from *Hansenula hostii* Y-2448; mAb, monoclonal antibody.

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normal mouse serum) were activated with PMA (100 ng/ml) for 10 min at 37°C. The cells were removed by centrifugation, and the supernatant was passed through a 0.22- μ m filter and then concentrated to <200 μ l with a Centricon-30 apparatus (M_r 30,000 cut-off; Amicon). The concentrate was washed twice with 2 ml of RPMI 1640 medium and then emulsified with complete Freund's adjuvant (first immunization) or precipitated in aluminum sulfate (second and third immunizations). Three mice were immunized i.p. at 2-week intervals. Between the second and third immunizations, spleen cells were removed and transferred i.p. into syngeneic animals. For the final booster injection, the mice were injected i.v. with whole peripheral blood lymphocytes to select for lymphocyte determinants. Four days later, spleen cells were removed and fused with the Sp2/0 myeloma cells as described (7).

Hybridoma supernatants were screened for the ability to differentially stain activated and unactivated peripheral blood leukocytes in an immunofluorescence assay. Briefly, cells that were activated with PMA (100 ng/ml; 10 min; 37°C) were labeled with fluorescein isothiocyanate (FITC) as described (6). The FITC-labeled activated cells and unlabeled unactivated cells were separately stained with hybridoma supernatant followed by a phycoerythrin-conjugated goat anti-mouse IgG second stage (Tago). The cells were washed, fixed in 1% paraformaldehyde, and then the activated and unactivated cells were combined for two-color flow cytometric analysis. Two hundred and eighty-eight hybridoma wells were analyzed. Five wells that gave identical staining patterns—a dramatic loss of staining on activated cells—were subcloned by limiting dilution analysis. The five mAbs against down-regulated antigens were designated DREG-55, -56, -110, -152, and -200 (all of the IgG1 subclass).

mAbs directed against human heterotypic cell adhesion molecule H-CAM (CD44) [H2-280 (IgG1) and Hermes-3 (IgG2a)], ICAM-1 [RR1/1 (IgG1)], CD11b [LM2/1 (IgG1)], CD18 [TS1/18 (IgG1)], LFA-3 [TS2/9 (IgG1)], T200 [L3B12 (IgG1)], Leu-8 (IgG2a; Becton Dickinson), and TQ1 (IgG1; Coulter) have been described (7, 21–25). mAbs against LAM-1 [LAM-1 (IgG1)] and ELAM-1 [CL3 (IgG1)] were kindly provided by T. F. Tedder (Dana-Farber Cancer Institute) and C. W. Smith (Baylor College of Medicine), respectively.

Western Blot Analysis. Lysates of freshly isolated peripheral blood mononuclear cells were prepared as described (25). In some experiments, lysates were precleared by three rounds of incubation with 100 μ g of mAb for 2 hr at 4°C followed by removal of antigen-antibody complexes with an excess of goat anti-mouse IgG-agarose (Sigma). Lysates were run under nonreducing conditions by SDS/9% PAGE and transferred to nitrocellulose by using a transblot apparatus (Bio-Rad). Western blot analysis was performed as described (25).

HEV Assay. The *in vitro* assay of lymphocyte binding to HEVs in frozen tissue sections has been extensively described (3, 4, 7, 26). Human lymphocytes show the same binding specificity on mouse and human lymphoid tissue (7, 26); thus, mouse peripheral nodes and Peyer's patches were used as examples of peripheral and mucosal lymphoid tissue, respectively. We have found, in agreement with Stoolman *et al.* (11), that centrifugation of lymphocytes through 25% Percoll to remove platelets greatly increases binding without affecting specificity. Cell binding was quantitated by first identifying HEVs in each field by dark-field illumination or by characteristic autofluorescence of the HEVs, and then counting cells bound to HEVs, as described (6, 7). Each data point within an experiment represents triplicate sections; each section with 100–250 PLN HEVs or 50–150 Peyer's patch HEVs is enumerated. Data are calculated as mean number of cells bound per individually scored HEV.

PPME Binding Assay. Lymphocyte binding of PPME, the phosphomonoester core from *Hansenula hostii* phos-

phomannan, has been described (11, 12). Briefly, human peripheral blood lymphocytes were pretreated with mAb (50 μ g/ml) for 30 min at 4°C, washed, and then exposed to a 1:100 dilution of fluorescein-conjugated PPME (generous gift of Steve Rosen, University of California, San Francisco) for 30 min at 4°C. The cells were washed, counterstained with phycoerythrin-conjugated goat anti-mouse IgG (second stage), and then analyzed by two-color flow cytometry for the ability of mAb to inhibit binding of the FITC-conjugated PPME.

DEAE-Dextran Transfection of COS Cells. The cDNA clones encoding ELAM-1 and LAM-1 were cloned from lipopolysaccharide-stimulated human umbilical vein endothelial cell cDNA and peripheral blood mononuclear cell RNA, respectively, by polymerase chain reaction by using primers corresponding to published 5' and 3' sequences (27–30). The cDNAs were subcloned into the expression vector CDM8 (30) and transfected into COS cells by DEAE-dextran as described (30). Forty-eight hours after transfection, COS cells were stained for immunofluorescence as analyzed by fluorescence microscopy.

RESULTS

Identification of Rapidly Down-Regulated Activation Molecules. We have previously shown that MEL-14 can be recovered in a largely intact form from the supernatant of mouse neutrophils activated with PMA (18). We postulated that this unusual and rapid down-regulation event would be conserved across species. We devised the following strategy to raise mAbs against rapidly down-regulated adhesion molecules on human leukocytes. Spleen cells from mice immunized with cell-free supernatant of PMA-activated human peripheral blood leukocytes were fused with SP2/0 myeloma cells. Hybridoma supernatants were screened for the ability to differentially stain activated and unactivated human peripheral blood leukocytes. Supernatants from five wells showed an 8-fold drop in mean fluorescence staining of

FIG. 1. Screening of DREG hybridoma supernatants on activated and unactivated human leukocytes. Unlabeled control (unstimulated) human peripheral blood leukocytes and PMA-activated human leukocytes labeled with fluorescein were stained with supernatants from uncloned DREG hybridomas followed by phycoerythrin (PE)-conjugated goat anti-mouse IgG (second stage). Stained cells were fixed and analyzed by two-color flow cytometry: fluorescein intensity (x axis) to distinguish activated and unactivated cells and PE intensity (y axis) to measure antibody staining. Hybridomas were selected for the ability of antibody-containing supernatant to stain activated and unactivated leukocytes differentially.

activated cells (Fig. 1). These hybridomas, designated DREG-55, -56, -110, -152, and -200, were subcloned. After preliminary characterization using supernatants from all five hybridomas, the DREG-55, -56, and -200 mAbs, which define distinct epitopes (see below), were partially purified and utilized for more detailed analyses.

Western Blot Analysis. Western blot analysis showed that the DREG-56 (Fig. 2, lane 1), -55 (lane 4), -200 (lane 5), -110 (not shown), and -152 (not shown) mAbs recognize a similar 72- to 77-kDa molecule under nonreducing conditions. Immunoprecipitation with the DREG mAb from 125 I surface-labeled cells showed a molecule of 80–85 kDa under reducing conditions (not shown). Preclearing lymphocyte cell lysates with the DREG-56 mAb removed the molecule reactive with the other DREG mAbs (lanes 6, 9, and 10, and data not shown) but not H-CAM (CD44) detected with the Hermes-3 mAb (lane 8). Preclearing with Hermes-3 did not remove the DREG-reactive species (data not shown). These results, taken together with fluorescence-activated cell sorting analysis of activated and unactivated leukocytes, suggest that the DREG mAbs recognize a single cell surface polypeptide.

The DREG mAbs Inhibit Human Lymphocyte Binding to PLN HEVs. Human peripheral blood mononuclear cells have been previously shown to bind to HEVs within frozen sections of human and mouse lymphoid tissue with identical specificity (6, 7, 26). Because of the limited accessibility of pathologically benign samples of human lymph tissue, mouse PLN and Peyer's patches were used in most of these studies. Pretreatment of human lymphocytes (3×10^7 cells per ml) with the DREG-55 and -56 mAbs inhibited binding to PLN HEVs by 85–95% of control medium but had no effect on binding to Peyer's patch HEVs (Fig. 3). The DREG-56 mAb was consistently more efficient at blocking binding than the DREG-55 mAb. The DREG-200 mAb showed only partial inhibition (50–65%) of binding to PLN HEVs, but like the other DREG mAbs had no effect on binding to Peyer's patch HEVs. The control mAb, H2-280, had no effect on lympho-

FIG. 3. The DREG mAbs inhibit human lymphocyte binding to PLN HEVs. Human peripheral blood mononuclear cells were pretreated with DREG-55, -56, or -200 or the class-matched control mAb H2-280 and were then incubated on freshly cut 10- μ m-thick sections of mouse PLN (solid bar) or Peyer's patches (hatched bar) for 20 min with mild rotation at 7°C. Mean number of lymphocytes binding per HEV was assessed as described. Results of six repeats from two separate experiments are presented as percent of control medium ($\pm$ SEM). Similar patterns of inhibition were obtained in two additional experiments with Ficoll-isolated but not Percoll-separated lymphocytes, although the overall binding per HEV was less when Percoll was not used.

cyte binding to either peripheral or mucosal HEVs. In further studies, DREG-56 mAb ($68\% \pm 4\%$ inhibition), but not H2-280, inhibited binding of human lymphocytes to HEVs within sections of pathologically benign human lymph node tissue. These experiments suggested that the gp80 antigen is a human PLN-specific lymphocyte homing receptor.

Previous studies have shown that most human B-lymphoblastoid cell lines are capable of binding to both peripheral and mucosal HEVs. Two exceptions are the KCA and LB25 cell lines, which bind selectively to mucosal HEVs and to peripheral HEVs, respectively (6). To determine whether differential expression of the DREG antigen could help account for this specificity, these lines were stained with the DREG mAb and analyzed by flow cytometry. LB25 cells have a 10-fold greater mode fluorescence staining with DREG mAb than KCA cells (Table 1). Furthermore, binding of LB25 to PLN HEVs is blocked by treatment with the DREG-56 mAb (data not shown). The DREG mAbs also stained the B-lymphoblastoid lines LBF and IBW4 (Table 1), which show dual binding specificity for PLN and Peyer's patch HEVs (6). In contrast, the lymphoma cell lines KW, CA46, and MOLT-4, which are unable to bind to HEVs (6), were essentially negative. Correlation of DREG staining and PLN HEV-binding ability is consistent with the gp80 antigen being an organ-specific homing receptor.

The gp80 Antigen Has Lectin-like Binding Activity. Rosen, Stoolman, and colleagues (10–12) have demonstrated that the MEL-14 antigen, a mouse PLN-specific homing receptor, is a lectin-like receptor whose activity can be inhibited by mannose 6-phosphate and the mannose 6-phosphate-rich mannan PPME (10–12). The ability of mouse lymphocytes to bind FITC-conjugated PPME is inhibitable by the MEL-14 mAb. Human lymphocytes also efficiently bind PPME (ref. 12; Fig. 4B). To determine whether the DREG antigen is involved in this activity, the DREG mAbs were tested for the ability to block human lymphocyte binding to PPME. Lymphocytes were pretreated with DREG mAb prior to exposure to FITC-conjugated PPME. Binding of the DREG mAb was revealed by a second stage goat anti-mouse IgG-conjugated phycoerythrin (i.e., see Fig. 4A). Two-color flow cytometry showed that DREG-56 (Fig. 4C) and DREG-152 (data not shown) almost completely inhibited PPME binding, DREG-55 (Fig. 4E) and DREG-110 (data not shown) showed only

FIG. 2. The DREG mAbs recognize a single molecular species. Cell lysates prepared from human mononuclear cells were mock-precleared (lanes 1–5) or precleared with DREG-56 mAb (lanes 6–10), run under nonreducing conditions on a SDS/9% polyacrylamide gel, transferred to nitrocellulose, and then analyzed by Western blot analysis. Primary mAbs were Hermes-3 (lanes 3 and 8) and DREG-56 (lanes 1 and 6), -55 (lanes 4 and 9), and -200 (lanes 5 and 10). Second stage control, alkaline phosphatase-conjugated goat anti-mouse IgG, is shown in lanes 2 and 7.

Table 1. DREG staining correlates with PLN HEV binding characteristics of human lymphoid cell lines

Cells		HEV binding*		DREG staining (mode fluorescence)†
Designation	Type	Binding	Specificity	
PBML	Peripheral blood	++++	PLN + PP	297
LB25	B lymphoblastoid	++	PLN	59
KCA	B lymphoblastoid	—	PP	5
LBF	B lymphoblastoid	+++	PLN + PP	331
IBW4	B lymphoblastoid	+++	PLN + PP	426
KW	Large cell lymphoma	—	Nonbinding	6
CRL	Burkitt lymphoma	—	Nonbinding	5
MOLT-4	T-cell lymphoma	—	Nonbinding	<0.5

PP, Peyer's patch.

*As reported in refs. 6 and 26.

†Mode fluorescence values (MFV) of staining with DREG-56 mAb minus MFV of control staining for each cell type. Control MFVs varied from 4.0 to 5.5.

partial blocking, and DREG-200 (Fig. 4D) had no blocking effect. Indeed, staining with DREG-200 showed a linear relationship with PPME binding of lymphocytes. Interestingly, the ability of the DREG mAb to inhibit PPME binding paralleled their relative efficiency in blocking binding of lymphocytes to PLN HEVs. The percentage of lymphocytes that stained with DREG mAb and with fluorescent PPME varied between donors (58–74%) but was always the same for each donor. In contrast, control mAb Hermes-3 did not block

PPME binding (Fig. 4F), and both PPME binding and non-binding lymphocytes stained with Hermes-3. These results suggest that the gp80 antigen is identical to or intimately associated with the PPME binding lectin on human lymphocytes.

DREG mAb Recognizes the Same Antigen Defined by LAM-1. Several laboratories have recently reported cDNA cloning of the putative human homologue of the MEL-14 antigen (27–30). The expressed product, termed LAM-1 by Tedder *et al.* (27), reacts with a LAM-1 mAb (T. F. Tedder, personal communication) and the leukocyte differentiation markers Leu-8 and TQ1 (ref. 30; T. F. Tedder, personal communication). Based on published sequences, we have cloned and expressed the LAM-1 cDNA in COS cells. COS cells transfected with the LAM-1 cDNA, but not with the structurally related ELAM-1 cDNA, react with LAM-1, Leu-8, TQ1, and DREG-55, -56, -110, -152, and -200 mAbs. Control mAbs L3B12, RR1/1, TS1/18, TS2/9, and LM2/1 did not stain LAM-1- or ELAM-1-transfected cells. CL3 mAb against ELAM-1 specifically stained ELAM-1-transfected cDNA.

DISCUSSION

We have raised mAbs against antigens that are rapidly shed from the cell surface of activated human peripheral blood leukocytes. One antigen, an 80-kDa glycoprotein defined by the DREG mAbs, fulfills the criteria for a lymphocyte homing receptor for PLN HEVs: (i) The DREG-56 mAb specifically blocks >90% of human lymphocyte binding to HEVs within peripheral lymphoid tissue without affecting binding to mucosal HEVs. (ii) Expression of gp80 correlates with the known HEV binding characteristics of several B-lymphoblastoid and lymphoma cell lines. (iii) gp80, like the PLN-specific MEL-14 antigen in the mouse, is dramatically down-regulated from lymphocytes and other leukocytes upon activation with PMA. (iv) gp80, like mouse MEL-14, appears to have lectin-like binding activity for PPME, an activity specifically involved in lymphocyte binding to PLN HEVs (10–12). (v) Preliminary immunohistology indicates that expression of gp80 appears limited to lymphocytes and other leukocytes and is developmentally regulated in a manner similar, if not identical, to mouse MEL-14 antigen (L. Picker, M.A.J., T.K.K., and E.C.B., unpublished observations). (vi) The DREG mAbs react specifically with the expressed product of LAM-1 cDNA, the putative human homologue of the MEL-14 antigen. Importantly, there has been no functional evidence that the LAM-1 cDNA or other cDNAs homologous to mouse MEL-14 cDNA (27–30) do indeed encode a human PLN homing receptor. Data presented here showing DREG inhibition of lymphocyte binding to HEV together with DREG staining of LAM-1-transfected COS cells indicate that the gp80 homing receptor and LAM-1 antigen are identical

FIG. 4. The DREG-56 mAb blocks lymphocyte binding of PPME. Human lymphocytes were pretreated with unlabeled mAbs, DREG-56 (C), -200 (D), -55 (E), or Hermes-3 (F), and then washed, incubated with fluorescein-conjugated PPME, washed again, and stained with phycoerythrin (PE)-conjugated goat anti-mouse IgG. Cells were analyzed by two-color flow cytometry for the ability of the test mAb (y axis) to block lymphocyte binding of PPME (x axis). Staining of lymphocytes with DREG-55 alone or fluorescein-conjugated PPME alone is shown in A and B, respectively.

and are the human homologue of the mouse PLN homing receptor, defined by MEL-14.

The three DREG mAbs characterized here appear to define at least three epitopes of gp80: DREG-56 blocks both PLN HEV binding and PPME binding; DREG-55 blocks PLN HEV binding almost as efficiently as DREG-56, but only partially blocks PPME binding; DREG-200 partially blocks HEV binding but does not block PPME binding. These results support previous work by Rosen, Stoolman, and co-workers (10–12) indicating that the lectin binding activity of the PLN homing receptor is directly involved in lymphocytes binding to PLN HEVs.

Another class of adhesion molecule/homing receptor involved in lymphocyte HEV interactions is H-CAM (the Hermes antigen, CD44; refs. 6 and 7). mAb Hermes-3 against H-CAM specifically inhibits binding of human lymphocytes to HEVs within mucosal but not peripheral lymphoid tissues or in inflamed synovium (7); however, polyclonal anti-H-CAM antiserum inhibits lymphocyte binding to mucosal, peripheral, and inflamed synovial tissue (7). Unlike DREG antigen and the mouse MEL-14 antigen, whose expression is limited to leukocytes, H-CAM is widely expressed by diverse cell types and is likely involved in many cell adhesion events (25). Recent cloning of human (21, 31) and primate (32) H-CAM and mouse (13, 14) and human (27–30) MEL-14 cDNAs has demonstrated that these two antigens belong to separate adhesion-related families: MEL-14 is a lectin-like protein, which is related to endothelial-leukocyte adhesion molecule 1 (ELAM-1; ref. 33) and the platelet and endothelial antigen GMP-140 (34), while H-CAM is related to cartilage link protein and proteoglycan core sequences (21, 31, 32). As suggested previously, the pan-HEV blocking activity of the polyclonal anti-H-CAM antiserum may be explained if H-CAM is a general adhesion-related protein that associates physically or functionally with other cell adhesion molecules, such as the PLN homing receptors defined by MEL-14 or DREGs (7).

The availability of mAbs against the human PLN homing receptor should provide insight into human lymphocyte traffic and development and into the importance of the PLN homing receptor in determining the metastatic behavior of leukemias and lymphomas. The human gp80 PLN homing receptor, like the mouse MEL-14 antigen, is also expressed by monocytes and neutrophils. Recently, the mouse MEL-14 antigen has been shown to be involved in neutrophil (15, 16) and monocyte (ref. 17; M.A.J. and E.C.B., unpublished data) extravasation in response to inflammation *in vivo* and binding to endothelium with frozen tissue sections *ex vivo*. Interestingly, expression of MEL-14 and Mac-1, a leukocyte integrin, is inversely regulated on mouse neutrophils after activation, suggesting that these molecules may mediate distinct but complementary adhesion events for neutrophil extravasation (16, 18). Thus, it seems likely that the gp80 DREG antigen will not be limited in function to lymphocyte homing.

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