Deficiency of Leukocyte Surface Glycoproteins Mo1, LFA-1, and Leu M5 in a Dog With Recurrent Bacterial Infections: An Animal Model

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A dog with severe recurrent bacterial infections, impaired pus formation, delayed wound healing, and severe persistent leukocytosis was the result of a mother-son mating. Assessment of leukocyte function revealed profound abnormalities in adherence-dependent activities including impaired granulocyte adhesion to glass/plastic surfaces or nylon wool, decreased granulocyte aggregation and chemotaxis, and diminished lymphocyte blastogenesis, but normal neutrophil oxidative activity, serum immunoglobulin, and complement levels. By immunofluorescence analysis, CD11b and CD18 monoclonal antibodies specific for the 155-kd α polypeptide of Mo1 (gp 155, 94) and the 94 kd β peptide common to Mo1, LFA-1 (gp 170, 94), and Leu M5 (p 150, 94) (surface molecules that promote leukocyte adhesion) failed to bind to unstimulated and A23187 calcium ionophore-stimulated granulocytes or mononuclear cells of the affected dog as compared with strong specific binding to canine control cells. The Mo1 glycoproteins were only barely detectable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of immunoprecipitates from lysates of 125I surface-labeled neutrophils from the affected dog as compared with intense bands seen with canine control cell precipitates. We conclude that this dog has a severe leukocyte surface glycoprotein deficiency syndrome that is similar, if not identical, to that recently recognized in humans. Dogs with deficiency of leukocyte Mo1, LFA-1, and Leu M5 expression may represent a useful animal model to characterize further the molecular basis for an inherited disorder in leukocyte effector function.

Three structurally related adhesion-promoting cell surface glycoproteins, Mo1, LFA-1, and Leu M5 are expressed by human leukocytes, as detected by murine CD11a,b,c and CD18 (World Health Organization nomenclature) monoclonal antibodies (reviewed in refs. 1 and 2). They are αβ heterodimers with an identical β subunit of 94 kd that is noncovalently associated with distinct α polypeptides of higher mol wt. The plasma membrane expression of these glycoproteins depends on the state of cellular activation and appears to be separately regulated despite sharing a common β subunit.3-5 Mo1 (gp 155, 94) has complement (C3bi) receptor type 3 (CR3) activity6-8 and plays a role in myeloid cell adhesion phenomena, including substrate adherence, chemotaxis, and aggregation.9-11 Lymphocyte function-associated antigen I (LFA-1; gp 170, 94) is expressed on phagocytes and lymphocytes and promotes lymphoid cell adhesion interactions that include lymphocyte proliferation and cytotoxic effector activity.12-16 Leu M5 (p 150, 94), the most recently characterized phagocyte surface protein, may represent a lectinlike surface molecule, the physiological significance of which is as yet unclear.17,18 Mo1 and/or LFA-1 glycoproteins (as detected by murine or rat monoclonal antibodies) have been identified in other species, including monkeys and other primates, mice, and dogs, indicating the conservation of these macromolecules during phylogeny.19,20

Recently, a combined Mo1, LFA-1, and Leu M5 leukocyte glycoprotein deficiency syndrome has been described in ~50 children who demonstrate an increased susceptibility to infections.4,5,21-32 This clinical syndrome is characterized by recurrent bacterial and fungal infections, progressive periodontitis, delayed umbilical cord separation, poor wound healing, and impaired inflammatory response. These patients have a persistent marked leukocytosis, with most of the cells being mature neutrophils, and profound abnormalities of adherence-dependent leukocyte functions. A moderate to severe deficiency of surface expression of leukocyte Mo1, LFA-1, and Leu M5 glycoproteins was noted in all cases, and a primary defect in the common β subunit of these glycoproteins is likely to underlie this syndrome.3

In this article, we report a leukocyte surface glycoprotein deficiency syndrome in the dog similar to that recognized in humans. The clinical features and the in vitro leukocyte functional abnormalities that characterize this disorder are described. CD11b and CD18 monoclonal antibodies reactive with canine Mo1, LFA-1, and Leu M5 facilitated our identification of the molecular basis of this animal's disease. In control dogs, the canine leukocyte surface glycoprotein complex was similar to that described in human and murine systems, and a severe deficiency of Mo1 and probably LFA-1 and Leu M5 glycoproteins was observed in the affected dog. This is the first report of an animal model of the human leukocyte Mo1, LFA-1, and Leu M5 deficiency syndrome.

MATERIALS AND METHODS

Case report. An 18-month-old female Irish setter cross-bred dog with a history of recurrent bacterial infections and persistent leukocytosis, first noted at the age of 2 months, was selected for study. The clinical presentations included deep skin wound infections, pododermatitis, superficial pyoderma, gingivitis, pneumonia, thrombophleb-
bits, and ascending osteomyelitis of the right hind limb. The infection sites usually showed a localized cellulitis with impaired pus formation and were often associated with a local lymphadenopathy. Episodes of infection were commonly associated with severe pyrexia (40.6°C), anorexia, and weight loss. Poor wound healing after minor trauma was also noted.

Cultures of the skin infections usually grew *Staphylococcus intermedius*. *Escherichia coli* was cultured from a aspirate of a metatarsal bone and from skin affected by pododermatitis. *Pseudomonas aeruginosa* grew in the fluid aspirate of a subcutaneous cyst that contained many bacteria but few phagocytes when examined cytologically. Bacterial infections responded very slowly to appropriate systemic antibiotic therapy and local skin care. Because of a persistent osteomyelitis, fever and lameness recurred several times within a week after drug withdrawal. The dog was thereafter treated with oral antibiotics. Fungal cultures and serum titers were negative, but *Trichuris vulpis* eggs were found on numerous fecal examinations. Antibody titers against distemper and parovirus infection appeared adequate following vaccinations with modified live virus vaccines.

Blood test results from the affected dog were collected for >1 year (Table 1) and revealed an extreme to moderate leukocytosis that persisted during apparently infection-free periods. The degree of the leukocytosis with WBC counts >208,000/μL was much greater than expected in normal dogs with similar bacterial infections. The persistent leukocytosis consisted predominantly of a mature neutrophilia, ranging from 21,000 to 188,000/μL with a concomitant eosinophilia and variable lymphocytosis and monocytoplasia. Morphologic examination of leukocytes by light microscopy as well as routine leukocyte cytochemistry were normal except for occasional hypersegmented granulocytes and atypical lymphocytes. A bone marrow biopsy demonstrated marked myeloid hyperplasia. Episodes of severe leukocytosis were usually associated with moderate anemia of inflammatory disease.

A mild polyclonal gammopathy due to an increased α2-protein fraction was also noted. Quantitative serum immunoglobulin concentrations and complement hemolytic activity (CH) levels were increased and consistent with an inflammatory disease. Episodes of leukocytosis were commonly associated with severe pyrexia (39.6°C), anorexia, and weight loss. Poor wound healing after minor trauma was also noted.

The studies were approved and in accordance with the school's guidelines for clinical research. An informed consent was received from the owner of the affected dog.

### Table 1. Hematologic Data of Affected Dog

|                  | Affected Dog* | Controls† |
|------------------|---------------|-----------|
| Hematocrit (%)   | 22-48         | 37-55     |
| Leukocytes (× 10⁹/μL) | 26-208       | 6-17      |
| Segmented neutrophils | 2.1-188    | 3-11.5    |
| Nonsegmented neutrophils | 0.1-2.4  | 0-3       |
| Lymphocytes      | 2.9-16.3      | 1-4.8     |
| Monocytes        | 0.6-14.2      | 0.1-1.3   |
| Eosinophils      | 1.4-15.6      | 0.1-0.8   |

*Data collected from 2 months to 1.5 years of age.
†Normal range for dogs, Veterinary Hospital, University of Pennsylvania, Philadelphia.

### Source and preparation of leukocytes

Leukocytes from the affected dog with recurrent bacterial infection and leukocytosis were studied several times simultaneously with leukocytes from one or more control dogs. Data obtained from the affected animal were compared with results collected from 12 dogs of various breeds (including Irish setters) that were healthy, as determined by physical examination and routine laboratory blood tests, and four dogs with typical infectious disease-associated leukocytosis. Venous blood was collected from a jugular vein in preservative-free heparin (10 IU/mL) or clot tubes and was processed within 4 hours. Polymorphonuclear leukocytes (PMNs) and mononuclear cells were prepared from heparinized venous blood by Ficoll-Hypaque centrifugation, gravity sedimentation with dextran and/or RBC lysis with hypotonic saline (0.2%) or 0.87% ammonium chloride at 4°C.31

### Monoclonal antibodies

Murine monoclonal antibodies specific for 155-kd α subunit of Mol (CD11b IgG1 anti-Mol α [904]) and the 94 kd β subunit common to all three leukocyte surface glycoproteins Mol, LFA-1 and Leu M5 (CD18 IgG2a IB4) were produced as previously described,7,8,2 and were generously provided by Dr James Griffin (Dana-Farber Cancer Institute, Boston) and Dr Samuel Wright (The Rockefeller University, New York), respectively. Additional murine monoclonal reagents specific for the α subunits of Mol (CD11b antibodies LM2/1,4,44,17), LFA-1 (CD1a antibodies TS1/12,22 TS1/22,22 and CLB-LFA-1/27,29) and Leu M5 (CD11c antibodies L29 and SHCL-33), and the common β subunit (CD18 antibodies 60.3,35,44,54 MHM2,38 and TS1/1832 from the CD11 and CD18 panels of the Third International Workshop and Conference on Human Leukocyte Markers were also used. Antibodies 3-1 (9-49²), anti-Mo2 (CD14, 116⁶), and anti-PAM 1 (p181⁴) were used as isotype-identical negative control reagents.

### Leukocyte function studies

The interactions of PMNs with various surfaces including cell adherence to plastic Petri dishes, glass coverslips, and nylon fibers were examined. In brief, PMNs (5 × 10⁷) suspended in Dulbecco's phosphate-buffered saline (PBS) were allowed to settle in a 35-mm plastic Petri dish at 37°C. After a 45-minute incubation, the fluid was removed, the dish was gently rinsed with 2 mL of the above buffer, and adherent cells were fixed in 2% (vol/vol) glutaraldehyde in PBS for 1 hour at 4°C. The number and morphology of the adherent cells were evaluated by phase-contrast microscopy. To study anchoring of cells to glass, freshly clotted blood was placed on a coverslip and incubated for 30 minutes as described for a neutrophil bacterial phagocytosis assay. The coverslips were prepared and evaluated as described above for the Petri dishes. Finally, heparinized whole blood was filtered through 70, 110, 150, and 190 ± 10 mg nylon fiber packed in the bottom 25 mm of a Pasteur pipette, and the percentage of PMNs adherent was calculated as described by McGregor and colleagues.48 Random migration and chemotaxis under agarose were measured according to Nelson and colleagues and described modifications,51 using zymosan-activated serum as an attractant.

Aggregation of PMNs was performed as previously described. In brief, canine neutrophils suspended in Hank's balanced salt solution (10²/mL) were added to aggregation cuvettes and warmed to 37°C. Aggregation was induced by the addition of phorbol myristate acetate (PMA) (125 ng/mL) and was recorded as an increment in light transmission on an arbitrary scale, with use of a platelet aggregometer. Aggregation of normal canine PMNs after incubation with anti-Mol (904) or a nonreactive control monoclonal antibody for 10 minutes at room temperature was also measured. The oxidative metabolic burst of resting and stimulated PMNs was assessed by the product formation of a redox reaction and generation of reactive oxygen derivatives. The reduction of nitroblue tetrazolium (NBT) by PMNs in suspension was examined by the spectrophotometric method of Baehner and Nathan using 0.81-
In flow cytometric studies, the oxidative product generation of the highly fluorescent 2',7' dichlorofluorescein from the nonfluorescent substrate 2',7' dichlorofluorescin was measured in single resting and PMA-stimulated cells according to Bass and co-workers.48

Lymphocyte proliferation response to phytohemagglutinin (2.5 or 5 μg/ml), concanavalin A (con A) (5 or 10 μg/ml), and pokeweed mitogen (PWM) (1 or 10 μg/ml) was measured in a microculture assay using canine hepatirinized whole blood.46

**Immunofluorescence flow cytometry.** Antigenic expression of Mo1, LFA-1, and Leu M5 leucocyte glycoproteins was investigated with mouse monoclonal antibodies against the α chain of Mo1 (CD11b, 904) and the common β chain (CD18, IB4) as previously described for human leukocytes.44 In selected experiments, surface expression of neutrophil glycoproteins was assayed after stimulation by A23187 calcium ionophore (1 μmol/L) for 15 minutes at 37°C.44 Indirect immunofluorescence flow cytometry was carried out by exposing PMNs to saturating concentrations of monoclonal antibodies for 30 minutes at 4°C and then, after washing, incubating them with a saturating concentration of fluorescein-conjugated goat antimouse immunoglobulin with added human IgG to block nonspecific binding mediated by Fc receptors. Immunofluorescence intensity of 5,000 leukocytes as a quantitative measure of relative antigen expression was analyzed on Coulter EPICS-C flow cytometer (Coulter Electronics, Hialeah, FL) using a logarithmic amplifier. To control for nonspecific binding of monoclonal anti-Mo1, LFA-1, or Leu M5 antibodies, parallel cell samples were exposed to isotype identical negative control monoclonal reagents.

**Immunoadsorption and electrophoretic analysis of leucocyte antigens.** Immunoadsorbed Mo1, LFA-1, and Leu M5 glycoproteins from lysates of 125I-surface labeled PMNs and mononuclear cells were analyzed by SDS-PAGE (7.5% gels). In brief, canine neutrophils and mononuclear leukocytes were surface labeled with 125I using the iodogen method.47 These labeled cells were solubilized in 0.01 mol/L of Tris-0.15 mol/L of NaCl buffer, pH 7.8, containing 1% NP-40, phenylmethylsulfonyl fluoride (PMSF) 1 mmol/L, iodoacetamide 10 mmol/L, EDTA 10 mmol/L, and 100 μg (each) of leupeptin, pepstatin, antipain, and chymotrypsin. 125I-labeled glycoproteins were isolated from NP-40 cell lysates by solid-phase immunoadsorption to immune complexes (murine monoclonal antibodies and affinity-purified F(ab')2 fragments of goat anti-mouse immunoglobulin) absorbed to polystyrol plastic.48 Washed immunoadsorbed antigen was solubilized in SDS sample buffer containing 2-mercaptoethanol and subjected to SDS-PAGE on 7.5% gels. Autoradiographs of dried slab gels were prepared, and the electrophoretic mobility of immunoadsorbed antigen was compared with that of protein standards.

**RESULTS**

Numerous in vitro leukocyte function studies were performed to characterize the leukocyte defect in the affected dog.

**Adherence.** Adhesion of the affected dog’s PMN cells to the surface of glass coverslips, plastic Petri dishes, and nylon fibers was markedly impaired as compared with that of control canine PMNs. PMN adherence as a function of nylon fiber weight is shown in Fig 1. The percentage of the affected dog’s PMNs adhering to the column was severely reduced at any nylon fiber weight between 70 and 190 mg. Complete adhesion of control dogs’ PMNs occurred at ≥150 mg nylon fiber, but was not achieved by PMNs from the affected dog. As indicated in Table 2, only few PMNs and mononuclear cells from the patient were adherent to the surface of glass coverslips or plastic Petri dishes. When examined by microscopy, the affected dog’s cells did not flatten, but had a round, refractile appearance, and were easily dislodged by a gentle stream of buffer, whereas normal cells were firmly spread on substrate.

**Table 2. Leukocyte Function Studies of Affected Dog**

| Function | Affected | Controls |
|----------|----------|----------|
| PMNs adherence* | | |
| (cells in ten fields) | | |
| Plastic Petri dish | 5 ± 4(3) | 78 ± 14(4) |
| Glass coverslip | 3 ± 3(3) | 64 ± 11(4) |
| PMN cell migration† | | |
| (arbitrary migration units) | | |
| Random migration | 1 ± 3(3) | 3 ± 1(3) |
| Chemotaxis (Zymosan) | 2 ± 1(3) | 8 ± 2(3) |
| NBT reduction§ | | |
| (OD) | | |
| Resting | 0.07 ± 0.03(3) | 0.05 ± 0.16(5) |
| Phagocytizing | 0.24 ± 0.07(3) | 0.19 ± 0.04(5) |
| Flow cytometry of DCF oxidation∥ | | |
| (log green fluorescence) | | |
| Unstimulated | 19.2 ± 16.6(3) | 8.3 ± 10.1(5) |
| PMA-stimulated | 158 ± 21(3) | 155 ± 22(5) |
| Lymphocyte blastogenesis¶ | | |
| (stimulation ratio) | | |
| Con A (5/10 μg) | 1.7/2.2 | 53/75 |
| PHA (2.5/5 μg) | 2.8/7.1 | 112/86 |
| PWM (1/10 μg) | 1/1 | 70/75 |

**Abbreviations:** PMNs, polymorphonuclear lymphocytes; NBT, nitroblue tetrazolium; DCF, 2',7' dichlorofluorescin; Con A, concanavalin A; PHA, phytohemagglutinin; PWM, pokeweed mitogen. Data are expressed as mean ± SD of (n), determination from affected dog or control dogs.

*Statistical difference between affected dog and controls, P < .05.†Adherent cells in ten microscopic fields (×100).‡Migration measured by arbitrary microscopic units.§Increased absorption at 515 nm caused by NBT reduction.∥Hydrogen peroxide production by PMNs measured by DCF oxidation; data expressed as the mean log green fluorescence.¶Representative experiment; stimulation ratio represents thymidine incorporation of stimulated over resting cells at two concentrations of stimulant.
**Chemotaxis.** The migration of the affected dog’s PMNs as well as the migration in response to the chemotactic factor, zymosan-activated serum, was poor as compared with that of canine control cells, as shown in Table 2. Indeed, the attractant failed to stimulate chemotactic migration of PMNs from the affected dog significantly.

**Aggregation.** Canine control PMNs aggregated readily on stimulation with PMA (Fig 2A and B). In contrast, no significant aggregation of PMNs from the affected animal occurred in the presence of PMA (Fig 2C).

**Oxidative burst reaction.** No abnormalities were detected in the granulocyte “burst reaction” of the affected dog (Table 2). The reduction of NBT by PMNs in response to latex particle phagocytosis and the oxidation of 2,7’ dichlorofluorescein by resting and PMA-stimulated cells from control and affected dogs was similar.

**Lymphocyte proliferation.** Mitogen-induced lymphocyte blastogenesis was markedly impaired in the affected dog as compared with controls (Table 2). Lymphocytes from the affected dog failed to proliferate in response to PWM, and only minimal responses were seen with con A and phytohemagglutinin (PHA).

**Expression of Mol, LFA-1, and Leu M5 canine leukocyte surface glycoproteins.** With a panel of murine monoclonal antibodies (including the CD11 and CD18 panels of the Third International Workshop on Leukocyte Markers) specific for human Mol, LFA-1, and Leu M5, normal and patient dog leukocytes were screened by immunofluorescence analysis for the expression of these glycoproteins. Among the reagents that cross-reacted with the PMNs of either normal dogs or control dogs with leukocytosis were CD11b antibodies 904 (shown in Fig 3), LM2/1, and 17 (each specific for epitopes on the α subunit of Mol1,3), and CD18 antibodies 1B4 (Fig 3), 60.3 MHM23, and TS1/18 (identifying epitopes on the β subunit polypeptide common to Mol, LFA-1, and Leu M52,7,11,18). Antibodies specific for the α subunits of LFA-1 (CD11a antibodies TS1/12, TS1/22, and CLB-LFA-1/2) or Leu M5 (CD11c antibodies L29 and SHCL-3) failed to bind to normal dog neutrophils (data not shown). In contrast to the reactivity of antibodies 904 and 1B4 for control dog cells, no specific antibody binding to the neutrophils of the patient dog was observed (Fig 3). Because exposure of human (and dog) neutrophils to degranulating stimuli in vitro results in an up-modulation of surface Mol antigen from cytoplasmic granular pools, we reexamined dog neutrophils after they were exposed to calcium ionophore A23187 to determine if patient dog neutrophils acquire detectable surface Mol expression from previously sequestered cytoplasmic stores. As shown in Fig 4, however, calcium ionophore A23187 treatment of patient dog neutrophils failed to stimulate the surface expression of Mol.

**Consistent with the results of immunofluorescence analyses, very little Mol αβ heterodimer was detectable in detergent lysates of 125I-labeled neutrophils or mononuclear cells from the patient dog as compared with readily apparent Mol antigen isolated from the cells of a normal control individual. This is shown in Fig 5: In the normal control, the polypeptides characteristic of Mol (p180, 96) could be isolated from neutrophil lysates by immunoadsorbence to antibodies 904 (lane 4) or IB4 (lane 6), whereas only faint bands are visible in corresponding lanes 3 and 5 representing immunoadsorbed antigen isolated from the neutrophils of the patient. In lane 6 (antigen from normal dog neutrophils adsorbent to the anti-β polypeptide antibody), an additional band of 160 kd was observed, which, based on experience in the human system, probably represents the α polypeptide of Leu M5. Moreover, the existence of an LFA-1 α polypeptide distinct from Mol α is suggested by doublet appearance of the broad 180-kd band apparent in lane 6. Neither of these additional polypeptides was found in corresponding lane 5 (patient dog antigen). Likewise, no detectable immunoadsorbed β-associated antigen was detectable in the lysate of 125I-labeled mononuclear cells from the patient (lane 10) relative to the three bands (180, 160, and 96 kd) isolated from the mononuclear cells of the normal control (lane 9). These results indicate that the leukocytes of control dogs express Mol and possibly LFA-1 and Leu M5 glycoproteins, but, in patient dog cells, the expression of these determinants is markedly deficient.

**Mol, which serves as an adhesion-promoting molecule of human neutrophils, performs a similar role in dog cells, as is suggested by the results shown in Fig 2: Anti-Molα antibody (antibody 904) attenuated neutrophil aggregation stimulated by PMA (tracing F) as compared with the normal response of neutrophils exposed to a negative control antibody (tracing E).**

**DISCUSSION**

In this study, we identified a dog with recurrent severe bacterial infections whose leukocytes demonstrated an impairment in adhesion-related interactions and a deficiency in the expression of Mol and related adhesion-promoting glycoproteins. The abnormalities in leukocyte function...
included significant decreases in neutrophil adherence to plastic, glass, and nylon wool, random migration and chemotaxis and aggregation in response to PMA, and a marked reduction in lymphocyte blastogenesis in response to mitogen stimulation (Table 2, Fig 1). Respiratory burst activity of the affected neutrophils was normal when latex particles or PMA was used as a stimulant (Table 2). A molecular basis for this impairment in adhesion-related leukocyte function was suggested by the deficient expression of leukocyte surface glycoproteins Mo1, LFA-1, and Leu M5, which, as shown in human and murine systems, contribute to leukocyte adhesion to substrates, chemotaxis, neutrophil aggregation, and to lymphocyte blastogenesis and cytotoxic function. The inhibitory activity of anti-Mo1 monoclonal antibody 904 on canine neutrophil aggregation stimulated by PMA (Fig 2) indicates a similar role for Mo1 in the dog system. Deficient expression of Mo1 and related molecules was demonstrated by immunofluorescence analysis in which murine monoclonal antibodies specific for the α polypeptide of human Mo1 (CD11b) or the β polypeptide common to Mo1, LFA-1, and Leu M5 heterodimers (CD18) failed to bind to the neutrophils of the affected dog but demonstrated specific
Fig 5. Immunoadsorption of Mol, LFA-1, and Leu M5 from NP-40 lysates of ¹²⁵I-surface-labeled neutrophils (GRAN) or mononuclear cells (MNCs) of the affected dog (lanes 1, 3, 5, 7, 10, 12) or a normal control animal (lanes 2, 4, 6, 8, 9, 11). Equivalent amounts of ¹²⁵I lysate from the affected dog or the control individual were incubated on a solid-phase immunoadsorbent consisting of IgG1 anti-Mol antibody, 904 (lanes 3 and 4), IgG2a anti-β antibody, IB4 (lanes 5, 6, 9, and 10), an IgG1 negative control antibody (lanes 1 and 2), or an IgG2a negative control antibody (lanes 7, 8, 11, and 12). Immunoadsorbed antigen was solubilized in sodium dodecyl sulfate (SDS) sample buffer containing β-mercaptoethanol and subjected to SDS-polyacrylamide gel electrophoresis on 7.5% slab gels. Numbers represent electrophoretic mobility of protein standards. For comparison, human Mol antigen consists of two polypeptides of 170 and 96 kd when electrophoresed on 7.5% gels (not shown).

cross-reactivity with the cells of control dogs (including animals with leukocytosis secondary to inflammatory disorders). Stimulation of the affected dog's neutrophils by calcium ionophore A23187 under conditions that up-modulated Mol surface expression in normal animals failed to induce detectable Mol expression (Fig 4). Moreover, detergent lysates of ¹²⁵I-labeled leukocytes from the affected dog were markedly deficient in their content of immunoadsorbable Mol glycoprotein (p180, 96 on 7.5% polyacrylamide gels) relative to that isolated from a normal individual (Fig 5). α Polypeptides of LFA-1 and Leu M5 that were discernible (albeit weakly) after immunoadsorption to anti-β subunit antibody from lysates of normal canine cells were absent from that of the affected dog (Fig 5).

The disorder in leukocyte function in association with deficient expression of Mol and related glycoproteins exhibited by this dog is similar to a clinical syndrome recently described in >50 human patients with leukocytosis and recurrent bacterial infections. In each case, the leukocytes of these patients demonstrated profound defects in neutrophil and monocyte adhesion to artificial and physiologic substrates, neutrophil and monocyte chemotaxis, neutrophil aggregation induced by a variety of inflammatory stimuli, as well as neutrophil and monocyte binding and phagocytosis of complement (C3bi-) opsonized particles. Certain individuals exhibited impairments in delayed hypersensitivity reactions, including lymphocyte blastogenesis in response to soluble recall antigens and mitogens, and in cytotoxic T lymphocyte and natural killing. The leukocytes of these patients were either partially or totally deficient in the expression of Mol, LFA-1, and Leu M5 glycoproteins, which, based on recent biosynthetic information, may be the result of biosynthesis of a defective 94-kd β polypeptide common to all three glycoproteins.

Reports demonstrating the inhibitory effect of glycoprotein-specific monoclonal antibodies on leukocyte function indicate that Mol (expressed by monocytes and neutrophils) is the surface receptor for C3bi (CR3) and also serves as an adhesion-promoting molecule of neutrophils and monocytes, whereas LFA-1 (expressed by all leukocytes) facilitates adhesive interactions involving lymphocytes and natural killer (NK) cells (the functional significance of Leu M5 has not been determined). Therefore, impairments in neutrophil and monocyte function (CR3 activity and adhesive interactions) exhibited by patient cells has been attributed to a deficiency in Mol, whereas defects in lymphocyte and NK cell activity (bysteric responses of lymphocytes; lymphocyte and NK cell cytotoxicity) are a consequence of a deficiency in LFA-1. By analogy, it is likely that the leukocyte functional abnormalities exhibited by the affected dog relate to the deficiencies in the expression of Mol and LFA-1.

Based on whether the deficiency in expression of Mol, LFA-1, and Leu M5 is partial (2.5% to 31% of the glycoprotein expression of normal leukocytes) or complete (<0.3% of normal Mol expression even after calcium ionophore stimulation), two forms of the disorder have been identified in humans, with good correlation between the level of glycoprotein expression and the degree of leukocyte functional impairment and the severity of the clinical syndrome. By the same criteria, the animal we studied would be classified as having a severe form of the disease with no Mol α polypeptide or common β peptide expression evident by immunofluorescence analysis and barely detectable amounts of Mol immunoadsorbed from cellular lysates.

Moreover, the clinical manifestations observed in this dog are unquestionably related to the profound adhesion dysfunctions of phagocytes in this disorder and are very similar to the clinical signs described in human patients with severe Mol, LFA-1, and Leu M5 deficiency syndrome. Bacterial infections of body surfaces such as skin and gingiva as well as osteomyelitis have previously been observed in human patients with this and other disorders that predispose to bacterial infections. Impaired pus formation and delayed wound healing appear to be more unique features of an abnormal inflammatory response in this disorder.

Of related interest are a series of reports by Renshaw and collaborators who described a neutrophil function defect...
in a male Irish setter dog and some of its offspring. Dogs with this so-called "canine granulocytopenia syndrome" had a history of omphalophlebitis after birth and subsequent episodes of recurrent bacterial infections including pyoderma, pododermatitis, lymphadenopathy, gingivitis, and osteomyelitis. These animals had a persistent, marked leukocytosis, with most cells being mature neutrophils. A neutrophil defect in bacterial activity due to a reduced glucose oxidation by the hexose monophosphate shunt was suspected, although these abnormal neutrophils showed increased capacity to reduce NBT dye. Unfortunately, most of these dogs died at a few months of age, and apparently none are available for further studies. Because the clinical features and routine laboratory test results appear to be identical with the disorder described here in an inbred Irish setter back-cross, however, the previously reported "canine granulocytopenia syndrome" may indeed be caused by a leukocyte Mol, LFA-1, and Leu M5 deficiency.

The study of family members of human patients with Mol, LFA-1, and Leu M5 deficiency suggests either an autosomal recessive or x-linked recessive mode of inheritance. The parents of affected offspring are clinically normal and generally exhibit normal leukocyte function. Certain parents and clinically normal siblings, however, express intermediate quantities of leukocyte surface glycoproteins (best detected after maximal in vitro stimulation of leukocytes), suggesting a heterozygous state.

The family history of the affected Irish setter mixed-breed dog suggests an autosomal recessive mode of inheritance, indicated because (a) the affected dog was a product of an Irish setter mother and her son (the father's father was of an unknown other breed), and (b) the parents and one female littermate were not afflicted by recurrent bacterial infections and had normal leukocyte counts, whereas another male littermate was reported to have a history of frequent bacterial infections. Preliminary leukocyte function tests and monoclonal antibody studies of mother and female littermate support an autosomal recessive inheritance with intermediate expression of leukocyte surface glycoproteins (data not shown). In addition, limited breeding studies of Irish setters with the previously described "canine granulocytopenia syndrome" also suggested an autosomal recessive mode of inheritance.

In conclusion, the comparative evaluations of the clinical features, the functional leukocyte abnormalities, and the molecular defect between the affected dog and the previously described human patients with Mol, LFA-1, and Leu M5 deficiency suggest that the patient has a leukocyte surface glycoprotein deficiency syndrome very similar if not identical to that recognized in humans. This is the first animal homologue of the leukocyte surface glycoprotein Mol, LFA-1, and Leu M5 deficiency. We are presently trying to establish an animal colony. The availability of an animal model for this disorder should prove useful in further characterizing the molecular basis and pathophysiology of deficient leukocyte glycoprotein expression and in developing new therapeutic approaches. In particular, functional aspects of these adhesion-promoting leukocyte glycoproteins and lack of expression could be studied in vivo, and gene replacement therapy could be developed in a large animal species with this defect.

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