Surface Membrane Expression by Human Blood Leukocytes and Platelets of Decay-Accelerating Factor, a Regulatory Protein of the Complement System


The decay-accelerating factor (DAF), an integral membrane protein of approximately 75,000 mol wt that regulates the stability of the C3 convertases of the classical and alternative complement pathways, was initially isolated from normal erythrocyte stroma and used to prepare a polyclonal antiserum. Previously, anti-DAF antiserum has been used to immunoprecipitate DAF from surface-labeled normal erythrocytes and to document the deficiency of DAF on the surface of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria, a condition in which erythrocytes express abnormal sensitivity to complement-mediated lysis. DAF has now been demonstrated by cytofluorography with anti-DAF F(ab')2 and fluoresceinated second antibody to be absent on the surface of the resting polymorphonuclear leukocytes (PMN), monocytes, lymphocytes, and platelets. Populations of PMN, monocytes, and platelets each exhibited a unimodal distribution of fluorescent staining, reflecting uniform cellular expression of DAF antigen, while the lymphocyte population had a skewed pattern of staining, indicating the heterogeneous expression of DAF antigen. For platelets, the shift in mean fluorescence channel observed with cytofluorographic analysis was minimal, but the presence of surface DAF on platelets was demonstrated by specific and saturable anti-DAF F(ab')2 binding. The DAF antigen, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of dithiothreitol-reduced anti-DAF immunoprecipitates prepared from surface-labeled, isolated populations of cells, presented a single polypeptide chain of approximately 84,000 mol wt for PMN and 75,000 to 80,000 mol wt for monocytes, T and B lymphocytes, and platelets. Thus, the complement regulatory protein, DAF, is expressed on the surface of all major types of circulating blood cells from normal donors. © 1985 by Grune / Stratton, Inc.

Decay-accelerating factor (DAF) is a membrane protein of approximately 75,000 mol wt that has been isolated from the erythrocyte (E) membranes of normal guinea pigs and normal humans. This protein, when solubilized, has the capacity to accelerate the decay of the C3 convertases of the classical and alternative complement pathways. Patients with paroxysmal nocturnal hemoglobinuria (PNH) have E that are abnormally sensitive to complement-mediated lysis (PNH-E) and are specifically deficient in the surface expression of DAF antigen, as documented by the marked absence of the antigen in immunoprecipitates of surface-labeled cells analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Type II PNH-E, which are three to five times more sensitive than normal E3 to complement lysis, are partially DAF deficient, while the type III PNH-E, which are ten to 15 times more sensitive to lysis, are totally DAF deficient on their surfaces, as demonstrated by their relative incapacities as intact cells to absorb anti-DAF IgG. After complement activation in vitro, abnormal amounts of C3b are detected on the surface of type II and type III PNH-E compared with normal E3, and the deficiency in DAF could explain the abnormal deposition of C3b on the surface of the PNH-E because of the impaired regulation of the function of C3 convertases. Evidence that the functional defect in PNH-E extends to precursor cells in the bone marrow and to circulating polymorphonuclear leukocytes (PMN) and platelets of PNH patients includes the abnormal sensitivity of these cells to complement-mediated lysis in vitro and suggests that the PNH defect arises as a clonal disorder from an affected bone marrow cell-derived line. The present studies establish the presence of DAF on the surface of normal PMN, monocytes, lymphocytes, and platelets and define the polypeptide structure of the DAF antigen on these cells as being a single chain of approximately 84,000 mol wt for PMN and in the 75,000 to 80,000 mol wt range for the other leukocytes and platelets.

MATERIALS AND METHODS

Reagents

The following reagents were purchased as noted: acrylamide, bisacrylamide, N,N,N',N'-tetramethylethylenediamine (TEMED), di-thiothreitol (DTT), SDS, and ammonium persulfate from BioRad Laboratories, Richmond, Calif; low mol wt standards, Sephadex G-25, Sephadex G-150 superfine, and cyagen bromide activated...
Sepharose from Pharmacia Fine Chemicals, Piscataway, NJ; N-formyl-l-methionyl-l-leucyl-l-phenylalanine, disopropylfluorophosphate (DFP), phenylmethylsulfonylfluoride (PMSF), and protein A Sepharose from Sigma Chemical Co, St Louis; Hanks’ balanced salt solution lacking calcium and magnesium (HBSS), and RPMI-1640 from GIBCO, Grand Island, NY; and sodium iodide (125I) from Amersham Corp, Arlington Heights, Ill.

Antisera

Polyclonal antisera was obtained from a rabbit immunized with solubilized DAF. IgG fractions of both the anti-DAF and the nonimmune rabbit sera were made by absorption to protein A Sepharose and elution of the bound IgG with 0.1 mol/L glycine-HCl buffer, pH 2.5. Eluted fractions containing protein, as determined by Folin assay, were pooled and adjusted to neutral pH with 1 mol/L TRIS (pH 10.6 at 4 °C). Although the anti-DAF IgG contained some IgG directed against glycophorin A, the anti-glycophorin A buffer, pH Sephanose and elution of the bound IgG with 0.1 mol/L glycine-HCl solubilized DAF. 2 lgG fractions of both the anti-DAF and the Antisera (125I) from Amersham Corp. Arlington Heights, Ill. protein A Sepharose from Sigma Chemical Co, St Louis; Hanks’ phosphate (DFP), phenylmethylsulfonylfluoride (PMSF), and pro-

TRIS, concentrated by negative pressure in a 75,000-mol wt cutoff into 0.1 mol/L Na acetate pH 4.4 buffer, by digestion for 16 hours at 37 °C with pepsin (Sigma Chemical Co) at a pepsin/IgG ratio of 1:100 (wt/wt). The mixture was adjusted to pH 7.0 with 1 mol/L TRIS, concentrated by negative pressure in a 75,000-mol wt cutoff collodion bag (Schleicher & Schuell, Keene, NH) and applied to a 2.5 × 90-cm G-150 superfine Sephadex column equilibrated in 0.150 mol/L NaCl, 0.005 mol/L sodium phosphate, 0.100 mmol/L EDTA. The completeness of the pepsin digestion and the purity of the F(ab’2) preparations obtained by chromatography were confirmed by SDS-PAGE with Coomassie Blue staining (Bio-Rad, Richmond, Calif), which revealed a band in the 100,000-mol wt region.

Cytofluorographic Analysis of Anti-DAF F(ab’2);

Because PMN, monocytes, and lymphocytes have distinct light-scattering characteristics, each cell type was gated for measurement of fluorescence intensity independent of the other cell types to permit the direct assessment of stained buffy coat leukocytes using an Ortho Systems 5DH-H cytofluorograph (Ortho Diagnostics, Westwood, Mass). Blood obtained from normal donors was diluted 1:11 with 0.50 mL of 0.24 mol/L citrate (pH 5.2) and 1.00 mL of Macrodex (Pharmacia). After the mixtures were allowed to settle at 37 °C for 30 minutes, the leukocyte-rich upper fraction was removed and sedimented at 375 g for five minutes at 4 °C. The leukocytes were resuspended in HBSS, washed three times in the same buffer by centrifugation, layered on Ficoll-Paque (Pharmacia) cushions and centrifuged for 40 minutes at 620 g at 4 °C. The mononuclear cell band and the PMN pellet were separately aspirated and each was diluted in HBSS, centrifuged at 375 g, and washed twice in HBSS. When necessary, contaminating erythrocytes were removed from the PMN fraction by hypotonic saline lysis and washing by centrifugation. To obtain lymphocytes, the mononuclear cells were exposed to 0.15 mol/L NH4CI lysing buffer, and this T cell-enriched population (97% of cells were positive for the T3 antigen > 95% viability. The mean fluorescence channel (MFC) was standardized for each analysis by adjusting the gain with rhodamine-conjugated or FITC-conjugated calf thymocyte nuclei (FluoroTrol-RF and FluoroTrol-GF, Ortho Diagnostics), respectively. Preliminary experiments, in which the first and second antibodies were titered, established that the amounts of antibody used in these studies were at saturating concentrations.

SDS-PAGE Analysis of Anti-DAF Immunoprecipitates Made From 125I Surface-Labeled Peripheral Leukocytes and Platelets

Five-milliliter portions of blood were diluted and mixed with 0.50 mL of 0.24 mol/L citrate (pH 5.2) and 1.00 mL of Macrodex (Pharmacia). After the mixtures were allowed to settle at 37 °C for 30 minutes, the leukocyte-rich upper fraction was removed and sedimented at 375 g for five minutes at 4 °C. The leukocytes were resuspended in HBSS, 0.1% BSA, for 20 minutes at 37 °C in Teflon containers, applied to Percoll (Pharmacia) in 0.15 mol/L NaCl, and centrifuged at 1,500 g for 25 minutes at 4 °C. The lower cell band that was formed by the lymphocytes was fractionated into T and B cell subsets by the addition of 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes to form rosettes with the T cells and sedimentation of the entire suspension through a Ficoll-Paque cushion. The nonrosetting lymphocytes at the interface of Ficoll-Paque, representing a B cell-enriched population (40% of the cells stained for the B1 antigen [Coulter, Hialeah, Fla] by indirect immunofluorescence), were washed into RPMI 1640 containing 10% heat-inactivated fetal calf serum (ΔFCS). The sheep erythrocytes adhering to the rosetted cells in the pellet were lysed by exposure to 0.15 mol/L NH4Cl lysing buffer, and this T cell-enriched population (97% of cells were positive for the T3 antigen [Ortho] by indirect immunofluorescence) was sedimented and resuspended in RPMI 1640 with 10% ΔFCS. The monocytes were obtained from a Ficoll-Paque mononuclear band that was enriched for adherent cells after surface labeling. All cell populations were counted on a Coulter counter (Model ZF, Coulter) and examined for trypan blue dye exclusion before labeling to assure > 95% viability.

PMN (2.5 × 107), mononuclear cells (7.5 × 107), T lymphocytes (2.1 × 107) and B-enriched lymphocytes (6.6 × 107) in 0.5 mL HBSS, and 7.3 × 107 platelets in 0.5 mL TBB were added to
EXPRESSION OF DAF ON BLOOD CELLS

individual borosilicate glass scintillation vials (R7491, Rochester Scientific, Rochester, NY) containing 160 μg of Iodo-Gen (1,3,4,6-tetracloro-3,6-diphenyl-glycoulir, Pierce Chemical Co, Rockford, Ill). One mCi of 125I was added to each reaction mixture, and the cells were labeled for 30 minutes at room temperature. Free 125I was removed by washing the cells three times in RPMI 1640 with 10% FCS using centrifugation at 375 g to pellet the cells after each wash. All cell types except the mononuclear cells were resuspended and lysed in 1 mL of 0.15 mol/L NaCl, 5.00 mmol/L Na phosphate, pH 7.5, 0.5% Nonidet P-40 (NP-40), 0.500 mmol/L DFP, and 0.050 mmol/L PMSF, and the insoluble debris was removed by centrifugation at 39,100 g at 4°C for 30 minutes. The labeled mononuclear cells were plated on plastic Petri dishes and incubated for one hour at 37°C. The nonadherent cells were subsequently washed away, and adherent cells were lysed by adding the NP-40 buffer to the plate. Then 250 μL of a slurry of approximately 250 μg of nonimmune F(ab')2, coupled to cyanoen bromide-activated Sepharose was added to the solubilized membranes of each cell type at 4°C, and each mixture was maintained in suspension for 30 minutes by agitation. The nonimmune F(ab')2 Sepharose complex was sedimented by 1,000 g for ten minutes at 4°C, and each supernatant was removed and reacted with 250 μL of a slurry of approximately 250 μg of anti-DAF F(ab')2, coupled to cyanogen bromide-activated Sepharose. Each mixture was kept in suspension for 30 minutes at 4°C, and the antigen-anti-DAF F(ab')2 Sepharose complex was pelleted by centrifugation. To analyze platelet DAF, nonimmune IgG and anti-DAF IgG were each prebound to protein A Sepharose for use in the sequential immunoprecipitation of solubilized membranes under conditions otherwise identical to those used for leukocytes. The nonimmune and immune Sepharose complexes were separately washed four times in 0.25% deoxycholic acid, 0.2% SDS, 0.15 mol/L NaCl, 0.05 mol/L TRIS-HCl, pH 9.0, diluted with an equal volume of sample buffer to give a final concentration of 10% glycerol, 2% SDS, 65 mmol/L TRIS-HCl, pH 6.8, and 10 μg/mL bromophenol blue, reduced in 40 mmol/L DTT at 60°C for ten minutes, and electrophoresed at 80 V on 6 cm x 13 cm x 1.5 mm 5% to 15% gradient polyacrylamide slab gels made with the Laemmli buffer system.19 Gels were fixed, stained in trichloroacetic acid–methanol– Coomassie Blue, dried, and autoradiographed at ~70°C with x-ray film (Kodak, Rochester, NY) and a Cronex enhancer (DuPont, Wilmington, Del).

Quantitation of DAF Antigenic Sites on Platelets

F(ab')2 fragments of nonimmune or anti-DAF IgG were radiolabeled with 125I and Iodo-Gen to specific activities of 2.7 x 105 and 1.4 x 105 cpm/μg, respectively. A fixed quantity of platelets, 1.8 x 107, was incubated with increasing doses of either nonimmune or anti-DAF IgG in 0.4 mL of TBB at 4°C with intermittent agitation. Duplicate 50-μL samples were removed from each reaction mixture after 30 minutes and layered onto 300 μL of an oil mixture (3:1 dibutylphthalate/dinonylphthalate, respectively) in polypropylene microfuge tubes (No. 72.702, Sarstedt, Princeton, NJ) at room temperature. The tubes were centrifuged for 30 seconds at 8,000 g in a microfuge (Model B, Beckman Instruments, Fullerton, Calif) to pellet the platelets. The tip of each microfuge tube was cut just above the platelet pellet, and both the tip of the tube and the upper portion containing the aqueous phase were counted to determine counts bound to platelets and free counts, respectively.9 The amount of nonimmune F(ab')2 binding versus input was plotted, and a line was fitted by the method of least squares analysis in order to calculate the amount of nonspecific F(ab')2 binding for each input. The amount of anti-DAF F(ab')2 binding versus input was plotted, and subtraction of the nonspecific binding yielded the specific 125I–F(ab')2 anti-DAF bound. These data were analyzed by Scatchard plots20 in order to calculate the number of polyclonal anti-DAF binding sites per platelet.

RESULTS

A representative cytofluorographic analysis (Fig 1) of the buffy coat leukocytes from a normal donor demonstrated that binding of anti-DAF F(ab')2 to each cell type increased the MFC above that produced by nonimmune F(ab')2 binding. This specific anti-DAF F(ab')2 binding to monocytes and PMN was unimodal and indicated the presence of DAF uniformly on the surface of each of these cell types. The lymphocytes exhibited a nonuniform distribution of DAF on the cell surface, consistent with the known heterogeneity of the lymphocyte population with respect to surface markers. Cytofluorographic analysis of platelets (Fig 2) from a representative normal donor revealed that anti-DAF F(ab')2 binding increased the MFC above that produced by nonimmune F(ab')2 binding. The distribution of DAF antigen on the platelet surface was unimodal, although the specific binding of anti-DAF F(ab')2 yielded a relatively small shift in the MFC. The cytofluorographic analyses of specific anti-DAF F(ab')2 binding to the buffy coat leukocytes and platelets from other normal donors are presented in Tables 1 and 2.

Because the surface expression of DAF antigen by platelets as analyzed by indirect immunofluorescence with a cytofluorograph was small relative to that of various leukocyte populations, the specific uptake by platelets of 125I-anti-DAF F(ab')2 was measured in a binding assay. The results for a representative normal donor (donor 10) indicated that binding of anti-DAF F(ab')2 was saturable (Fig 3), and a Scatchard plot of these data (Fig 3, inset) allowed the calculation of 2,240 molecules of polyclonal anti-DAF F(ab')2 bound.

![Fig 1. Cytofluorographic analysis ofuffy coat leukocytes for DAF antigen. Upper left panel: pattern of light scatter by leukocytes with notation of gating for lymphocytes (region 1), monocytes (region 2), and PMN (region 3). Remaining panels show the fluorescence due to nonimmune F(ab')2 binding (—) and anti-DAF F(ab')2 binding (-----) of lymphocytes, monocytes, and PMN of donor 1.](image-url)
per platelet. Similar results were found for three other normal donors (Table 3).

The molecular forms of the DAF antigen present on the various leukocyte types from donors were analyzed by SDS-PAGE of anti-DAF F(ab')2 immunoprecipitates made from PMN, monocyte, and T and B cell-enriched lymphocyte populations that had been surface labeled with 125I and subsequently solubilized. A representative autoradiograph (Fig 4) of the SDS gel documented that there was a single band present in the anti-DAF immunoprecipitate of each cell type that was not precipitated from the same cell type of the nonimmune F(ab')2. Similar SDS-PAGE analysis of 125I surface-labeled platelets from three donors revealed that anti-DAF F(ab')2 precipitated DAF from platelet membranes as a single band (Fig 5). The apparent mol wts of the DAF immunoprecipitated from different cells of a single donor showed some variation, being 83,000 for PMN, 78,000 for monocytes, 79,000 for T cells, and 79,000 for B cells. There was also some variation among donors for the same cell type, although these variations were at the limits of detection by this analysis. The DAF from PMN of all donors studied (n = 4) averaged a slightly higher mol wt (84,000) than the DAF from other leukocytes (n = 4) and platelets (n = 3), which generally had a mol wt in the 75,000 to 80,000 range.

The B cell-enriched lymphocyte population contained only 40% B cells, as indicated by positive indirect fluorescence with monoclonal anti-B1 and cytofluorographic analysis. To determine if the DAF was actually expressed by the B cells, the lymphocytes from three donors were obtained from Percoll gradients, reacted with both rabbit anti-DAF F(ab')2, and mouse monoclonal anti-B1, and then with FITC-labeled goat anti-mouse, and biotin-coupled goat anti-rabbit (Vector Laboratories, Burlingame, Calif) and avidin-rhodamine (Vector). The results of a representative analysis showed that all the B1-positive cells also bear DAF antigen (Fig 6); the cells that moved away from the y axis, indicating B1 expression, also moved away from the x axis, demonstrating the presence of DAF antigen.

**DISCUSSION**

A functional assay of the capacity of soluble DAF to augment the decay of the classical complement C3 convertase initially allowed the purification of the DAF protein by sequential chromatographic steps.1,2 Studies by Hoffmann more than ten years ago had identified decay-accelerating activity for classical guinea pig C3 convertase in the aqueous phase of butanol-extracted human,21,22 guinea pig, and rabbit23 erythrocytes, and limited fractionation procedures had suggested that there were at least two different decay accelerating activities in the extract. It is now known that both solubilized 75,000-mol wt DAF1,2 and 250,000-mol wt C3b receptor24,25 (CR1) have decay-accelerating activity toward C3 convertases and that they are immunologically distinct.2 In addition, in situ these membrane proteins have different predominant activities: DAF is primarily responsible for the accelerated decay of the alternative pathway C3 convertase when the convertase is on the same cell,4 whereas CR1,26,27 and probably not DAF,27 has decay-accelerating activity for classical C3 convertase assembled on a bystander cell. The I-cofactor activity for C3b deposited on an autologous cell and for soluble C3b is provided by CR1 and not DAF.4,27

A significant in vivo function for DAF in protecting human E membranes has been established by the demonstration that DAF antigen is deficient on the surface of E from patients with PNH,3,4 an acquired disease in which the affected E have an abnormal sensitivity to lysis by activation of autologous complement both in vivo and in vitro.28,29 The occurrence in

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**Table 1. Cytofluorographic Analysis of DAF Antigen on Peripheral Blood Leukocytes Expressed as Mean Fluorescence Channel**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Nonimmune F(ab')2</th>
<th>Anti-DAF F(ab')2</th>
<th>Nonimmune F(ab')2</th>
<th>Anti-DAF F(ab')2</th>
<th>Nonimmune F(ab')2</th>
<th>Anti-DAF F(ab')2</th>
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</thead>
<tbody>
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<td>320</td>
<td>80</td>
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</tr>
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<td>2</td>
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patients with PNH of aplastic anemia, nonlymphoid leukemias, and abnormalities of coagulation suggests that there may be coincident abnormalities of other blood cell types, including at least granulocytes and platelets. Both myeloid and erythroid precursors in bone marrow cells from patients with PNH are more sensitive to complement-mediated lysis. Unfractionated leukocytes from PNH donors contained cells that were abnormally sensitive to complement lysis with a \( {^{51}}\text{Cr} \) release assay; a granulocyte fraction from a Ficoll-Hypaque gradient was also abnormally sensitive to complement lysis as measured by \( {^{51}}\text{Cr} \) release, which in turn was correlated with the enhanced deposition of C3b on the surface of these cells. Excessive \( {^{51}}\text{Cr} \) release and \([^{14}C]\) serotonin release provided functional evidence that platelets from PNH donors were abnormally sensitive to complement-mediated lysis and release reactions, respectively; in addition there was enhanced C3b deposition on the surface of the PNH platelets. In contrast, as assessed by \( {^{51}}\text{Cr} \) release non-glass-bead-adherent lymphocytes from the circulating blood of a PNH donor were not abnormally sensitive to autologous serum complement. In addition, when a T lymphocyte-enriched fraction from PNH donors was tested for sensitivity to complement-mediated lysis using rabbit serum as a source of complement and \( {^{51}}\text{Cr} \) release as a measure of lysis, there was no abnormality, and enhanced deposition of C3b was not detected on the surface of these T cells after sensitization with anti-I and reaction with human serum.

Preliminary studies have demonstrated DAF determinants on normal platelets by immunoprecipitation of the protein and on platelets and lymphocytes by inhibition of decay-accelerating activity for an alternative pathway convertase formed on these cells by

**Table 2. Cytofluorographic Analysis of DAF Antigen on Platelets Expressed as Mean Fluorescence Channel**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Nonimmune F(ab')2</th>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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<td>8</td>
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</tbody>
</table>

**Table 3. DAF Antigen Sites on the Surface of Normal Platelets**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Molecules of Polyclonal Anti-DAF Per Platelet</th>
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<td>3,600</td>
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<tr>
<td>3</td>
<td>1,547</td>
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<tr>
<td>9</td>
<td>2,074</td>
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<td>10</td>
<td>2,240</td>
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</table>

**Fig 3.** Enumeration of DAF antigenic sites on the surface of nonactivated platelets from normal donors. 125I-anti-DAF F(ab')2, binding (•—•); 125I-nonimmune rabbit F(ab')2, binding (A—A); calculated specific anti-DAF F(ab')2, binding (O—O). Inset: Scatchard analysis of the anti-DAF F(ab')2 data. Assuming a predominant binding affinity, the values for the lowest two F(ab')2 inputs (23 and 63 ng) have been deleted because they were off the scale. A line fit by least squares of the experimental points gives an x intercept of 8.7 ng, which calculates to 2,240 polyclonal F(ab')2 anti-DAF binding sites per platelet.

**Fig 4.** Autoradiographs of anti-DAF F(ab')2 immunoprecipitates prepared from the solubilized membranes of enriched populations of various types of circulating leukocytes that had been surface labeled with \( ^{125} \). The precipitates were electrophoresed on a 5% to 15% polyacrylamide slab gel after reduction with 40 mmol/L dithiothreitol. The mol wt standards—phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), \( \alpha \)-lactalbumin (14,400)—were iodinated and stained with Coomassie Blue. The position of the albumin band on the dried gel and the position of the remaining standards were determined from the dried gel and marked on the autoradiograph. NI, nonimmune; sDAF, anti-DAF.
Introduction of anti-DAF. In the present studies, DAF antigen has been identified on the surface of normal platelets, PMN, monocytes, and lymphocytes by cytofluorographic analysis and by immunoprecipitation of surface-labeled cells. Although the anti-DAF raised against DAF isolated from normal erythrocyte membranes reacts with glycophorin A, its monospecificity for the leukocytes and platelets was established by the single band of protein, DAF antigen, precipitated in the surface labeling experiments (Figs 4, 5). The cytofluorographic analysis demonstrates that DAF is expressed on the surface of relatively nonperturbated leukocytes (Fig 1, Table 1) since this technique allows the analysis for fluorescence of separate cell populations without physical fractionation of the cells. Routine cell isolation procedures have been shown to enhance markedly the expression of the other membrane protein with complement C3 convertase regulatory activity, the C3b receptor (CR1). Although the monocytes, PMN, and platelets expressed a unimodal distribution of fluorescence, indicating uniformity for each population (Figs 1, 2), the pattern of fluorescence expressed by lymphocytes was skewed, suggesting that the different subpopulations of lymphocytes express variable amounts of DAF per cell. The double-staining analysis definitively indicated that B cells do bear DAF antigen (Fig 5), while the presence of this antigen on T cells was confirmed by immunoprecipitation. Since T cells dominate the cytofluorographic analysis of the combined peripheral blood lymphocyte population, the heterogeneity of DAF antigen expression presumably resides in this population.

The relatively small shift in MFC induced by the indirect fluorescence using anti-DAF binding to platelets necessitated confirmation of the presence of DAF antigen by measuring directly the specific binding of radioactive anti-DAF F(ab')2. Scatchard analysis of the binding data (Fig 3) demonstrated saturable binding and a number of anti-DAF binding sites that ranged from 1,547 to 3,600 (Table 3) for four normal donors. Because polyclonal anti-DAF was used, it was not possible to calculate the number of DAF molecules per platelet, as this determination would require assay with monoclonal anti-DAF or a functional ligand. The relatively small number of polyclonal anti-DAF F(ab')2 bound per platelet (Table 3) would be at the theoretical limits of detection by the cytofluorographic methods used and is consistent with the slight change in the MFC documented for platelets after anti-DAF binding (Fig 2, Table 2).

The results of the anti-DAF immunoprecipitation of surface-labeled leukocytes (Fig 4) and unactivated platelets (Fig 5) indicate that the DAF antigen on all these cells is a single peptide chain, as has been described for erythrocytes. The immunoprecipitate...
made from the B cell-enriched lymphocyte population had only 40% B cells, as defined by cells bearing the Bi antigen, with the majority of the remaining cells presumably being T cells. Despite this complex population of cells, only a single DAF band was detectable by immunoprecipitation (Fig 4). Direct evidence that the B lymphocytes express DAF was obtained from the cytofluorographic analysis of lymphocytes doubly stained with mouse Bi and rabbit anti-DAF (Fig 6). The mol wt of DAF from PMN averaged 84,000, whereas the DAF from other cell types and platelets was usually in the 75,000- to 80,000-mol wt range. In a similar type of analysis, DAF immunoprecipitated from normal erythrocytes had a mol wt of about 75,000, whereas the soluble erythrocyte DAF that has been isolated by chromatography has a mol wt of 70,000. Extensive studies of CR1 have documented that different donors have different mol wt forms of CR1 and that these forms are genetically determined. In addition, the CR1 antigen from PMN is about 5,000 higher in mol wt than the CR1 from the erythrocytes and monocytes from the same donor.

The finding that DAF antigen on nonerythroid cells has a similar molecular form, namely a single polypeptide chain in the 75,000-mol wt range, as on the erythrocyte, makes it possible that the somatic mutation that apparently occurs in a bone marrow precursor cell and gives rise to DAF-deficient erythrocytes may produce other DAF-deficient progeny due to the same molecular defect. This could also be the basis for the enhanced susceptibility to complement-mediated lysis that has been well documented for granulocytes and platelets from PNH donors.

ACKNOWLEDGMENT

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Surface membrane expression by human blood leukocytes and platelets of decay-accelerating factor, a regulatory protein of the complement system

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