Molecular Characterization of Antigenic Polymorphisms (Ond$^a$ and Mart$^a$) of the $\beta_2$ Family Recognized by Human Leukocyte Alloantisera


We show that the previously described alloantisera Ond and Mart, which recognize the alloantigens Ond$^a$ and Mart$^a$, react with polymorphic variants of $\alpha_\l$ and $\alpha_\m$ subunits of the $\beta_2$ integrin family (CD11a and CD11b molecules). This was shown by testing the alloantisera in a monoclonal antibody-specific immobilization of leukocyte antigens, immunoprecipitation, and immunofluorescence assay against cells from normal donors and from patients with leukocyte adhesion deficiency ($\beta_2$ integrin deficient). To elucidate the molecular basis of the Ond$^a$ and Mart$^a$ alloantigens, RNA was isolated from mononuclear leukocytes derived from individuals of known serologic phenotype. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to amplify the entire coding region of the $\alpha_\l$ and $\alpha_\m$ mRNAs. The Ond$^a$ antigen was found to be due to a G2466C substitution in the DNA coding for the $\alpha_\l$ subunit, which predicts an Arg766Thr amino-acid polymorphism. The Mart$^a$ antigen was also found to be due to a single nucleotide substitution (G302A) in the DNA coding for the $\alpha_\m$ subunit, which predicts an Arg61His amino acid polymorphism. Using allele-specific restriction enzyme analysis, the association between point mutations and phenotypes was confirmed. The localization of these alloantigens on integrin molecules further illustrates the polymorphic nature of this class of proteins. Whether the polymorphisms influence the adhesive capacity of the leukocyte integrins remains to be investigated. © 1996 by The American Society of Hematology.

HEMATOPOIETIC CELLS express various adhesion molecules, many of which are structurally related and belong to a superfamilies, the integrins. Within this superfamilies, three families can be distinguished: the LeuCAM, the VLA, and the cytoadhesin family. The LeuCAM family comprises molecules involved in virtually all cell-cell interactions of the immune system. It consists of three members sharing a common $\beta$ subunit of 95 kD ($\beta_2$ or CD18-antigen) noncovalently associated with the 3 different $\alpha$ subunits of 180 kD ($\alpha_1$, CD11a antigen), 165 kD ($\alpha_4$, CD11b antigen), and 150 kD ($\alpha_3$, CD11c antigen), respectively. The CD11a and CD11b (VLA-1) are expressed on all leukocytes; the CD11b (C3bi receptor) and CD11c antigens are expressed on granulocytes, monocytes, and natural killer (NK) cells.

Many members of the integrin family have been shown to express polymorphic antigenic determinants that are of clinical importance. Pischel et al. have described an antigenic polymorphism of the $\alpha_\m$ subunit of the $\beta_2$ integrin family. The antigen was detected with a serum (E27) from a multiple transfused systemic lupus erythematosus (SLE) patient. In platelets, the $\beta$ subunit of the cytoadhesin family (p2, or glycoprotein [GP] IIIa) expresses the HPA-1 (Zw), HPA-4 (Yuk/Pen), HPA-6 (Ca/Tu), HPA-7 (Mo), and HPA-8 (Sr) alloantigens and the private alloantigen Gro. The $\alpha_\m$ subunit of this family (platelet GPIIb) expresses the HPA-3 (Bal) and the recently identified Max alloantigen. The $\alpha_\m$ subunit of the VLA family (VLA-2) carries the HPA-5 (Br) alloantigens. The molecular basis of these platelet alloantigens was found to be due to single amino-acid polymorphisms.

In the present study, we show that the alloantisera Ond and Mart, which recognize the alloantigens Ond$^a$ and Mart$^a$, react with polymorphic variants of $\alpha_\l$ and $\alpha_\m$ subunits of the $\beta_2$ integrin family, respectively. The antigen detected by serum Ond was found to be identical to that recognized by serum E27. Similar to what is known for the platelet alloantigens, the leukocyte alloantigens Ond$^a$ and Mart$^a$ are associated with single nucleotide substitutions leading to amino-acid dimorphisms.

MATERIALS AND METHODS

Sera

Serum Ond was obtained from a male patient suffering from aplastic anemia and has been described previously. The patient had been transfused with packed red blood cells and platelet concentrates from more than 700 random donors. Despite these transfusions, he never produced antibodies against HLA or platelet-specific antigens. Moreover, it was shown that he had a markedly prolonged survival of a skin graft from an HLA nonidentical donor (35 days) and from his HLA-compatible brother (70 days). In the immunofluorescence test, his serum was found to strongly react with lymphocytes, monocytes, and granulocytes. It did not react with the lymphocytes from 4 of 49 donors. The serum also contained anti-NA2 alloantibodies. The inheritance of the target antigen of serum Ond was studied in the family of one of the workers in the lab.

Serum Mart was obtained from a multiparous woman who had no history of giving birth to babies with clinically apparent neonatal alloimmune neutropenia. This serum was reactive in the immunofluorescence test with granulocytes, monocytes, and a subset of lymphocytes.

Serum E27 was kindly provided by Dr K.D. Pischel (University of California, San Diego, CA). Serum CLB120 is a pool of human sera containing a mixture of antibodies against different HLA-anti-
gens. As negative control, we used pooled serum of nontransfused male donors with blood group AB.

Monoclonal Antibodies (MoAbs)

The following MoAbs were used: CLB-CD7 (CD7), W6/32 (directed against HLA class-I antigens), CLBLFA1/1 (CD18), CLBLFA1/2 (CD11a), Bear-1 (CD11b), and HC1/1 (CD11c). Bear-1 was kindly provided by Dr C. Figdor (Netherlands Cancer Institute, Amsterdam, The Netherlands).10 HC1/1 was a gift of Dr E. Sanchez-Madrid (Madrid, Spain).31 The other antibodies were produced in our own laboratory and were clustered during the Third International Workshop on Leukocyte Differentiation Antigens.32

β2 Integrin-Deficient Patients

Two unrelated patients with congenital leukocyte adhesion deficiency (LAD) donated cells for our study. The cells of patient BH express a low but detectable amount (~10%) of LFA antigens.33 Patient VI is a newly diagnosed β2 integrin-deficient girl, who died from sepsis shortly after birth. No β2 integrin antigens were detectable on her cells.44 Immunofluorescence tests were performed according to standard methods with PFA-fixed cells.45

Cells

Granulocytes and mononuclear cells were isolated from heparinized blood by Ficoll-Isoaque density centrifugation (d = 1.077 g/mL). Lymphocytes and monocytes were further purified by elutriation. Morphologic examination showed greater than 90% purity.

MoAb Immobilization of Leukocyte Antigens (MAILA)

MAILA assay was performed according to Kiefel et al,36 with minor modifications. In a typical experiment, 106 packed cells (previous experiments showed that large numbers of cells were necessary to obtain clear results) were incubated in 50 μL of diluted MoAb (ascitic fluid samples, diluted 1:50 in phosphate-buffered saline [PBS] containing 0.1% [vol/vol] bovine serum albumin [BSA]) for 30 minutes at 37°C. Then, without washing, 50 μL of human serum was added and the mixture was incubated for another 30 minutes at 37°C. The cells were washed 3 times in PBS/0.2% BSA and, after pelleting, were solubilized for 30 minutes at 4°C in 100 μL of Tris buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.4) supplemented with 0.5% (vol/vol) NP40. To remove unsolubilized material, the lysate was centrifuged for 30 minutes at 12,000g at 4°C. The top 50 μL of the supernatant was collected and added to 200 μL of a washing buffer (TRIS-buffer supplemented with 0.5% [vol/vol] NP40, 0.05% [vol/vol] Tween 20, 0.2% [wt/vol] BSA, and 0.5 mmol/L CaCl2). One hundred microliters was transferred in duplicate to a 96-well microtiterplate (DYNatech, Alexandria, VA), precoated with goat-antimouse-Ig (7.5 μg/mL GAM; Jackson, Westgrove, PA) in 0.1 mol/L NaHCO3/Na2CO3 pH 9.6 overnight at 4°C. The plate was incubated for 60 minutes at 37°C, followed by 30 minutes at 4°C. After extensive washing at 4°C, the wells were incubated with 100 μL of alkaline phophatase-conjugated rabbit-anti-human-IgG (Jackson) diluted 1:500 in washing buffer for 2 hours at 4°C, followed by another washing cycle. Finally, the bound conjugate was visualized by adding Sigma 104 R Alkaline Phosphatase substrate (Sigma Chemical Co, St Louis, MO) dissolved in diethanolamine buffer, pH 10. To inhibit endogenous alkaline phosphatase activity, levamisole was added to the substrate buffer. The absorbance was measured on a titertec reader.

Immunoprecipitation

In a typical experiment, 106 cells, radiolabeled with 121I with iodogen as catalyst, were incubated with 50 μL of MoAb (ascites 1:100 diluted) or 100 μL of human serum for 30 minutes at 4°C. In some experiments, the cells were incubated with both MoAb and human serum. The cells were washed 3 times in PBS/0.5% BSA and solubilized in 500 μL of immunoprecipitation buffer (IPB; containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% NP40 [wt/vol] supplemented with 1 mmol/L phenylmethyl sulfon fluoride [PMSF], 0.02 mg/mL trypsin inhibitor, and 5 mmol/L EDTA as protease inhibitors) for 30 minutes at 4°C. The lysate was centrifuged for 30 minutes at 12,000g. The supernatant was preclayed by end-over-end incubation for at least 6 hours with 25 mg of control CNBr-sepharose 4B-beads (Pharmacia, Uppsala, Sweden) suspended in IPB. These control beads were activated and blocked according to the manufacturer’s instructions without coupling of any protein. From the preclayed lysate, the antibody-antigen complexes were precipitated with polyclonal goat-antimouse-Ig or monoclonal anti-human-IgG covalently coupled to Sepharose 4B-beads by end-over-end incubation for 3 hours at 4°C. In sequential immunoprecipitation, the supernatant was incubated with 5 μL of ascitic fluid 1:10 or 25 μL of human serum, again precipitated with anti-Ig coupled to Sepharose beads. This procedure was repeated 3 times. Finally, the supernatant was preclayed with human serum and incubated with MoAbs and vice versa. The isolated antigens were analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography.

Isolation of RNA and cDNA Synthesis

Total cellular RNA from mononuclear leukocytes was prepared by extraction with guanidine thiocyanate and density gradient centrifugation in cesium chloride. cDNA first-strand synthesis was performed on 1 μg of total RNA in 25 μL of a solution containing random hexamers, 1 μL of each deoxynucleotide (10 mmol/L), 1 μL of dithiothreitol (DTT; 0.1 mol/L), RNase inhibitor, and 1 μL of Superscript reverse transcriptase II (200 U; GIBCO-BRL, Gaithersburg, MD). The mixture was incubated at 42°C for 60 minutes. The reaction was inactivated by heating at 95°C for 10 minutes, and 50 μL of water was added to bring the volume to 75 μL.

PCR Amplification of cDNA and Sequence Analysis

Suitable oligonucleotide primers were used to amplify the entire coding region of the α/β3 subunit cDNAs. The PCR was performed on 1 μg of cDNA, 100 pmol of each primer, 10 mmol of each dNTP, and 2 U of Taq DNA polymerase (Promega Corp, Madison, WI) diluted in a buffer recommended by the manufacturer in a total volume of 50 μL in a DNA thermal cycler (Model 480; Perkin Elmer-Cetus, Norwalk, CT). An initial denaturation cycle at 95°C for 5 minutes was followed by 35 cycles with denaturation at 95°C for 1 minute, annealing at 55°C for 1.5 minutes, and extension at 72°C for 2.5 minutes. The nucleotide sequence of the overlapping PCR products was determined directly with the cycle sequencing kit with 32P-labeled (Amersham, UK) primers, following the instructions of the manufacturer (GIBCO-BRL).

PCR-Allele-Specific Restriction Enzyme Analysis (PCR-ASRA)

Bsp1286I ASRA to distinguish Ond+ from Ond- . A 709-bp segment of the α2 cDNA containing the dimorphism was amplified with oligonucleotide primers LS5 (2283-2303) 5′tagatctATTTC TTGGTCGTGG3′ (extended with EcoRI and HindIII restriction sites, respectively). Ten microliters of the PCR product was digested with the restriction endonuclease Bsp1286I (New England Biolabs, Beverly, MA) and subsequently size-separated on a 2%
Therefore, it was possible to test this serum in the MAILA. The specificity of the assay was shown using a serum withotide primers M720-700 agarose gel, stained with ethidium bromide, and visualized with instructions of the manufacturer (New England Biolabs). The reknown specificity for HLA class recognized by the CD1 la and CD18 MoAbs, ie, the LFA-1 obtained when the two sera under investigation tested against the binding of CD11 or CD18 MoAbs (data not shown).

Absorbance values obtained in the MAILA assay using lymphocytes, monocytes, and granulocytes. The three cell types were incubated simultaneously with the sera under investigation (AB, Ond, or Mart) and the MoAbs CLB-LFA1(1CD11a), CLB-LFA1/2 (CD11a), Bear-1 (CD11b), or CLB120 (CD1 IC). The indicated values represent the mean. The number of experiments performed for each combination is given between parentheses. Statistical significance (compared with AB control) was calculated by using the Student's t-test. 

Abbreviation: NT, not tested.

Aci I ASRA to distinguish Mart** from Mart**. The oligonucleotide primers M36-56 5'TGCCACCTCTCTCCAGGTT3' and M720-700 5'CTTCACCGTGACCTGGT3' were used to generate a PCR product of 685 bp from cDNA. An aliquot (10 μL) of the PCR product was subjected to Aci I digestion according to the instructions of the manufacturer (New England Biolabs). The resulting fragments were electrophoresed and visualized on a 2% agarose gel with ethidium bromide and UV light.

**RESULTS**

GP Recognized by Ond Antibodies

In the immunofluorescence test, it was shown that binding of the antibodies present in serum Ond did not interfere with the binding of CD11 or CD18 MoAbs (data not shown). Therefore, it was possible to test this serum in the MAILA. In this assay, a positive signal is only obtained when a human serum contains antibodies against the same GP as the MoAb. The specificity of the assay was shown using a serum with known specificity for HLA class I antigens. This serum (CLB120) produced a much higher signal (mean absorbance value 2.163 ± 0.071 ± SD, n = 5) than an AB serum (0.096 ± 0.045) when tested against an MoAb (W6.32) specific for HLA class I molecules. As expected, no positive results were obtained when the two sera under investigation tested against an irrelevant control MoAb (0.204 ± 0.071 and 0.196 ± 0.103, respectively). As shown in Table 1, Ond serum contained antibodies reactive with the same GP complex (present on monocytes, granulocytes, and lymphocytes) as that recognized by the CD11a and CD18 MoAbs, ie, the LFA-1 complex. Ond serum did not react with the CD11b and CD11c MoAbs. The results obtained with the MAILA strongly suggested that the Ond serum was reactive with the LFA-1 GP.

To confirm this finding, sequential immunoprecipitation experiments with Ond serum were performed. As shown in Fig 1, GPs of the same molecular weight (MW) were immunoprecipitated from surface-labeled lymphocytes with Ond serum as with anti-LFA-1 (CD11a), ie, 180 kD and 95 kD. After the lymphocyte lysate was precleared with the anti-LFA-1 MoAb (CLB-LFA1/2), no antigens were precipitated by Ond serum, but cell lysate precleared with an MoAb against a non-LFA antigen (CLB-CD7) still contained the 180-kD and 95-kD proteins recognized by Ond serum. Similarly, lymphocyte lysate precleared with serum Ond no longer contained LFA-1 complex antigens, which could be immunoprecipitated with the MoAbs (Fig 1). Ond serum reacted in the immunofluorescence assay with lymphocytes and granulocytes obtained from two unrelated LAD patients. However, when these cells were radiolabeled, Ond serum did not precipitate the characteristic bands of 180 and 95 kD, as seen with normal donor cells. These results indicated that Ond serum contained other antibodies in addition to the Ond antibodies. Indeed, we have previously shown that antibNA2 antibodies were also present in this serum.7 Pischel et al previously described a serum that immunoprecipitated LFA-1 from lymphocytes of 95% of individuals tested. This serum, E27, did not precipitate LFA-1 from a lysate of lymphocytes obtained from the person who donated Ond serum (data not shown), and Ond serum did not immunoprecipitate any GPs from a lysate of the lymphocytes obtained from patient E27 (K. Pischel, personal communication, 1989).

Molecular Basis of Ond

Because the results of immunochemical and serological tests with Ond serum indicated that the epitope was located on GP α L, we examined the nucleotide sequence of the α L cDNA derived from lymphocyte RNA obtained from patient Ond. Six overlapping fragments that spanned the entire length of α L cDNA were amplified by PCR. The nucleotide sequence of the PCR fragments was determined and compared with the previously published sequence of α L cDNA. Three differences were observed: (1) a C to T change at position 1882, predicting no amino acid change (Leucine 571); (2) an inversion of nucleotides T2072 and A2073, predicting a tyrosine (TAC) to isoleucine (ATC) change at
position 635; and (3) a G to C substitution at position 2466, predicting an arginine to threonine transformation at position 766 of the mature GP (Fig 2). These findings prompted us to determine the αc cDNA sequences of five other people whose leukocytes had been Ond-phenotyped (2 Ond" and 3 Ond"). The C1882T (Leu571) and Tyr(TA C)635Ile(ATC) changes were observed in all donors studied, whereas the G2466C (Arg766Thr) substitution was present in the αc cDNA of Ond" donors only.

The G2466C mutation abolishes a target site for the restriction endonuclease Bsp1286I, which cleaves at 5'-GAG-CCC-3' (Ond") but not at 5'-CAGCCC-3' (Ond") sequences (Fig 3A). As can be seen from Fig 3B, Ond" can be clearly distinguished from Ond' by cDNA typing with Bsp1286I. Moreover, it was possible to identify individuals heterozygous for the Ond" antigen (Fig 3B, lane 3). Because the genomic structure of LFA-1 is not yet known, we were not able to generate a PCR-ASRA using genomic DNA.

**Ond Phenotype**

We next studied the association of the G2466C (Arg766Thr) polymorphism with the serologically determined Ond phenotypes in a family (phenotyped by MAILA; Fig 4). Bsp1286I ASRA was performed with PCR products derived from lymphocyte RNA from the members of one family. Full concordance between serology and PCR-ASRA was obtained (data not shown). Moreover, three members of this family appeared to be heterozygous for the G2466C (Arg766Thr) dimorphism. The family of propositus Ond was not studied because they were not available for study.

**GPs Recognized by Mart Serum**

In the immunofluorescence assay, the binding of the allo-antibodies in Mart serum was not blocked by either CD11 or CD18 MoAbs, which made it possible to test this serum in the MAILA assay. As shown in Table 1, Mart serum reacted in the MAILA assay with a complex recognized by CD11b and CD18 antibodies, i.e., the C3bi-receptor (CR3). Immunoprecipitation studies were performed to verify this finding. In initial experiments, no positive results were obtained. Because of the positive results with Mart serum in the MAILA assay, we modified our procedure and performed immunoprecipitation under experimental conditions similar...
to those used in the MAILA assay, i.e., preincubation of intact cells with both anti-CR3 MoAb and serum. As shown in Fig 5, Mart serum (lane 2) in the presence of this MoAb precipitated granulocyte proteins with a similar MW (165 and 95 kD) as that precipitated by anti-CR3 antibody (lanes 1 and 3). This result was not due to the cross-reaction of the antihuman-Ig Sepharose beads with murine Ig, because no proteins were immunoprecipitated when the MoAb specific for CR3 was incubated in combination with human AB serum (Fig 5, lane 4). Further evidence of the specificity of the Mart serum for β1 integrin antigens was provided by the finding that, when Mart serum was tested in the immunofluorescence assay against lymphocytes and granulocytes from the two unrelated LAD patients, no reactions occurred.

**Molecular Basis of Mart**

To identify the molecular basis of the Mart” antigen, five overlapping segments of the αM cDNA derived from the Mart” donor were amplified by PCR with suitable oligonucleotide primers. Comparison of the nucleotide sequence of the PCR products with the published sequences of αM showed a G to A change at position 302, predicting an arginine to histidine substitution at position 61 of the mature GP (Fig 6). The two previously described polymorphisms of CD11b/αM cDNA, i.e., the absence of glutamine at position 478 and a C2969T transition (predicting a Pro950Leu substitution), were not detected in the cDNA of the Mart” donor.

**Mart” Typing by Aci I ASRA**

We took advantage of the loss of a target site for the restriction endonuclease Aci I (5’-CCGC-3’) by the G302A substitution to type for the Mart” antigen. As can be seen in Fig 7A, a 685-bp segment in the aminoterminus of the αM cDNA was amplified and subsequently digested with Aci I. In the Mart” allele, the PCR product (685 bp) was cleaved into three fragments of 272 bp, 266 bp, and 147 bp, but in the Mart” allele, due to the absence of one of the Aci I target sites in the 685-bp PCR product, only two fragments of 538 bp and 147 bp were generated. In Fig 7B, the ASRA results obtained with PCR products derived from a Mart”, a Mart”, and an apparently heterozygous donor are shown. The Mart” members of the Mart family appeared to be
The antigen recognized by serum Mart is expressed on granulocytes, monocytes, and a subpopulation of T lymphocytes. The calculated gene frequency is 0.906. Serum Mart as well as two other sera with identical specificity were derived from multiparous women. The antigen recognized by serum Ond is expressed on granulocytes, monocytes, and T and B lymphocytes. The serum reacted with the cells of 45 of 49 donors.

Our first indication of the GP specificity of the antisera was obtained from MAILA experiments. Ond' and Mart' antigens were immobilized by CD18 MoAbs, indicating that the alloantibodies reacted with GPs of the β2 family. The finding that Ond' antigen was immobilized only by anti-α1 and Mart' only by anti-αM antibodies strongly suggested that the sera were reactive with epitopes on the two different α-chains, α1 and αM, respectively. The sera were not reactive in the immunoblot technique (data not shown).

Further evidence for the specificity of the sera was obtained by immunoprecipitation. Ond' serum immunoprecipitated a 180/95-kD complex, corresponding to the MW of a molecule precipitated by an anti-LFA-1 antibody. The identity of this complex was finally proven by sequential immunoprecipitation with anti-LFA-1 antibodies. Immunoprecipitation studies also showed that the antigen recognized by serum E27, described by Pischel et al, is identical to the Ond' antigen. Mart' serum immunoprecipitated the C3bi receptor (CR3). The latter serum did not react with the cells from heterozygous for the G302A mutation (Fig 8). Full concordance was obtained between serotyping and genotyping for the Mart' antigen (data not shown).

**DISCUSSION**

At present, most of the polymorphic antigen systems identified on platelets have been biochemically localized. It is remarkable that at least 8 of these systems are localized on molecules belonging to the integrin supergene family; HPA-1, -3, -4, -6, and -9 and the private platelet antigens Gro' and Va' are present on cytoadhesins and HPA-5 on VLA-2. Although numerous leukocyte antigens have been identified and characterized with murine MoAbs, most non-HLA leukocyte antigen systems defined by human alloantibodies have not yet been biochemically characterized. We have shown that the NA system is located on the Fcγ receptor III (CD16 antigen). Stroncek et al and Goldschmeding et al identified the NB1 antigen on a 58- to 64-kD GP located on the neutrophil plasma membrane and in secondary granules. In the present study, we show that the previously described polymorphic leukocyte antigens Ond' and Mart' are located on the α1 subunit of the LFA-1 complex and the αM subunit of the C3bi receptor, respectively.

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two unrelated LAD patients. Positive immunoprecipitation results with Mart serum were obtained only when the labeled cells were simultaneously incubated with both the serum and the CR3 MoAb. The binding of the MoAb apparently stabilizes the LFA complex and conserves the Mart' antigenic site during solubilization.

We have elucidated the molecular basis of the Ond' and Mart' alloantigens by RT-PCR followed by direct nucleotide sequence analysis. Both alloantigens were found to be associated with single nucleotide substitutions resulting in amino-acid dimorphisms of the corresponding \( \alpha \) chains of the \( \beta_2 \) integrin family. Results from DNA typings of phenotyped individuals and family studies suggest that the amino-acid dimorphisms control the expression of the Ond and Mart epitopes on the \( \alpha_L \) and \( \alpha_M \) molecule, respectively. However, this should be definitely proven by the construction and analysis of the reactivity of allele-specific forms of the \( \alpha \) chains with the Ond and Mart alloantisera in expression systems. It is not yet possible to develop a typing technique at the genomic DNA level, because the genomic organization of the \( \alpha_L \) and \( \alpha_M \) chains is not yet known. However, based on our findings, such genotyping strategies can be easily designed as soon as the genomic structures have become available.

Thus, similar to that of platelet alloantigens, the molecular basis of the leukocyte alloantigens Ond' and Mart' has been found to be due to single nucleotide substitutions, leading to amino-acid dimorphisms. The localization of two more alloantigen systems on integrin molecules further illustrates the polymorphic nature of this class of proteins. Binding of antibodies to these alloantigens may affect adhesion via such receptors. Antibodies against HPA-1a (Zwa') have been shown to inhibit fibrinogen binding to activated platelets. The survival of skin grafts on patient Ond was remarkably prolonged. This was possibly due to the alloantibodies against LFA-1 present in his serum, which may have blocked leukocyte-leukocyte interactions in the grafts. Thus far, in vitro functional studies with Ond serum have not shown any
functional effects. However, these results may have been due to the low titer of the antibodies in the serum. Because murine LFA-1 MoAbs can prevent graft-versus-host disease, it is possible that the Ond antibodies may have been active in vivo. Thus, by immortalizing the alloantibody-producing cells of patients such as Ond or Mart, it may be possible to produce human MoAbs against β2 integrins, which may be clinically useful in preventing graft rejection or graft-versus-host disease.

In conclusion, we located the leukocyte antigens Ondα on the α5 chain of the LFA-1 complex and Martα on the α6 chain of the C3bi receptor and established the molecular genetic basis for both leukocyte antigens. This shows that antigenic polymorphisms also occur in the β2 integrin family of integrins.

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Molecular characterization of antigenic polymorphisms (Ond(a) and Mart(a)) of the beta 2 family recognized by human leukocyte alloantisera

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