

IMPAIRED F_c-MEDIATED MONONUCLEAR PHAGOCYTE
SYSTEM CLEARANCE IN HLA-DR2 AND
MT1-POSITIVE HEALTHY YOUNG ADULTS*

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The increased frequencies of the B cell alloantigens DR2, DR3, and MT1 in systemic lupus erythematosus (SLE) have suggested that genes influencing either disease susceptibility or expression are located in or near the major human histocompatibility HLA complex on chromosome 6 in man (1-3) and have raised the possibility that some immunologic abnormalities that occur in disease could be associated with the genetic marker per se rather than be solely a reflection of the disease process.

Marked impairment of Fc receptor-dependent mononuclear phagocyte system (MPS) clearance occurs in SLE (4, 5). Normals with the HLA-B8/DR3 phenotype may also have delayed MPS clearance (6). Since MPS-clearance dysfunction might reflect at least in part an intrinsic cellular defect, we examined the Fc receptor-mediated MPS clearance and in vitro monocyte function in disease-free young adults with the other HLA antigens seen with increased frequency in SLE. Our results indicate that normals with a haplotype containing either HLA-DR2, -MT1, or -B8/DR3 have impaired MPS clearance compared with their other normal controls. Fc-dependent phagocytosis by circulating monocytes was also impaired in the DR2 and MT1 groups.

Materials and Methods

Subjects. 10 female and 29 male disease-free Caucasian adults participated in the MPS-clearance study after giving written informed consent. 4 female and 13 males were donors for in vitro studies. No subject had a family history of SLE or other autoimmune disease sharing the HLA associations seen in SLE. Protocols for all studies were approved by the institutional Committees on Human Rights in Research.

Histocompatibility Antigen Determination. Histocompatibility testing (HLA) for serologically detectable antigens of the A, B, C, DR, and MT series was performed on isolated mononuclear cells and nylon wool-separated B lymphocytes as described (1).

MPS Fc Receptor-mediated Clearance Studies. Fc receptor-mediated MPS studies were done by previously described techniques (4, 5).

Cell Preparation. Mononuclear cells were separated by density centrifugation on Ficoll-Hypaque and purified for aggregate-binding and rosette-formation assays by adherence to

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plastic surfaces (7). 1×10^6 peroxidase-positive cells in RPMI 1640 with 20% gammaglobulin-free fetal calf serum (FCS) (Gibco Laboratories, Grand Island, NY) were plated onto a disposable plastic dish (Falcon Labware, Oxnard, CA) that had been precoated overnight with heat-inactivated FCS containing $<5 \mu\text{g/ml}$ of fibronectin. After 60 min at 37°C in a humidified 5% CO_2 atmosphere, and washes to remove nonadherent cells, disodium EDTA, 0.2% in Hanks' balanced salt solution (HBSS), was added and the plates placed at 4°C for 20 min to remove adherent cells. The adherent cells were harvested after gentle action with a rubber policeman, washed, counted, and resuspended in HBSS.

For phagocytosis of IgG-sensitized erythrocytes, mixed mononuclear cells obtained by Ficoll-Hypaque centrifugation were stained with peroxidase, and $2.5\text{--}5 \times 10^5$ peroxidase-positive cells were added to each duplicate incubation mixture. The peroxidase-staining characteristics of circulating mononuclear cells gradients did not vary according to immunogenetic subgroups (DR2 positive: $30 \pm 6\%$ [mean \pm SD]; DR2 negative: $34 \pm 11\%$).

Aggregated-IgG-binding Assay. IgG model immune complexes, covalently cross-linked with bis-diazotized benzidine, were made in one uniform batch and sized by column chromatography. Sucrose-density centrifugation demonstrated predominantly dimeric complexes with $\sim 10\%$ larger aggregates. Aliquots were stored at -70°C and iodinated by the iodine monochloride technique every 2 wk. Specific activity of the iodinated aggregates ranged from 10 to 42×10^7 dpm/nmol. IgG molarities were calculated assuming a molecular weight of 160,000 (8).

Triplicate samples of $3\text{--}5 \times 10^5$ monocytes in HBSS with 0.2% bovine serum albumin and 0.1% sodium azide were incubated at 37°C for 1 h with varying concentrations of iodinated aggregates both with and without a large excess of unlabeled aggregates (~ 100 -fold excess). Cell-bound and free aggregates were separated by centrifugation of cells through a layer of phthalate oils (dibutyl phthalate/bis [2-ethylhexyl] phthalate, 1.5:1.0) with a Beckman Microfuge B (Beckman Instruments, Inc., Palo Alto, CA). Aliquots of the supernatants were removed for gamma counting to determine the concentration of free aggregate. The microfuge tubes were pinched off below the aqueous organic interface and the sedimented cell-bound radioactivity determined after cutting the tip.

At least 98% of mononuclear cells labeled with ^{51}Cr passed through the phthalate oils after centrifugation. $<0.001\%$ of radiolabeled aggregate sedimented in centrifuged cell-free suspensions. At least 80% of saturably bound IgG aggregate was displaced by 90–120 min after further incubation with a large excess of cold aggregate. Specific binding, in molecules of IgG per monocyte, was calculated after subtraction of nonspecific binding at each ligand concentration (8).

Antibody-sensitized Erythrocyte (EoxA) Rosettes. Bovine erythrocytes were incubated with rabbit IgG anti-bovine erythrocyte antibody (Cappel Laboratories Inc., Cochranville, PA) for 1 h at 37°C . The amount of antibody used gave maximum rosette formation with peripheral blood monocytes; it was a fourfold dilution of the minimal agglutinating titer.

EoxA Phagocytosis. EoxA were prepared as above with the addition of $5 \mu\text{Ci}$ of ^{51}Cr during sensitization. Duplicate samples of mixed mononuclear cells for each experimental point were combined with varying ratios of EoxA per monocyte, ranging from 5 to 100. The mixture was centrifuged at $44 g$ for 5 min and incubated at 37°C for 1 h. Noninternalized EoxA were lysed with ammonium chloride solution. After four washes, the cell pellet was counted in a gamma counter to determine mononuclear cell-associated chromium. Overall uptake rates were expressed as the mean number of erythrocytes phagocytosed per monocyte per hour after subtraction of an unopsonized Eox control.

Data Analysis. MPS-clearance half-time values were determined by nonlinear regression (9). The saturable binding of IgG aggregates was analyzed by Scatchard plots (8). The best fit of the experimental points was determined by least squares linear regression. Phagocytosis of EoxA at various erythrocyte/monocyte ratios was assessed by Lineweaver-Burke analysis (10). The experimental points were fit by least squares linear regression; the apparent maximum velocity (V_{max}) and apparent Michaelis-Menten constant (K_m) were determined as the reciprocals of the Y and X intercepts, respectively.

The MPS-clearance values for the different subject groups were analyzed by the chi-square test for homogeneity. The upper 95% confidence limit of the control group in each instance defined the upper limit of normal. The Fisher exact test was used to evaluate all 2×2 tables. Central tendencies were compared by the Mann-Whitney U test. Mood's test of dispersion was used to compare the distribution of experimental points in the relevant populations (11).

Results

MPS Fc Receptor-mediated Clearance. 8 normals had the B cell alloantigen DR3 and 15 were positive for DR2. Two of these subjects were positive for both. Because of the association of B cell alloantigens DR2 and DR3 with SLE lupus, the normal population was analyzed for differences in MPS clearance in either of these two groups. Subjects positive for DR2 were distinguishable from both non-DR2/non-DR3 normals ($P = 0.033$; Fisher exact test, one-tailed) and all normals including those with DR3 ($P = 0.049$). Subjects positive for DR3 were not distinguishable from either non-DR2/non-DR3 normals or all others including DR2. Division of the normal group into those positive and negative for either MT1, MT2, or MT3 did not reveal any significant differences.

Previous work by Lawley and associates (6) had suggested that Fc receptor-mediated MPS clearance in normal subjects with the HLA-B8/DR3 phenotype was prolonged. Accordingly we identified the five subjects in our population with both antigens and considered them as a separate group. Since MT1 is associated with DR2, we also considered the possibility that subjects who were MT1-positive but DR2-negative might share MPS-clearance characteristics more closely resembling DR2 than their non-DR2 colleagues. Accordingly, we divided our normal population into four groups: DR2-positive, MT1-positive/DR2-negative, B8/DR3, and other (Fig. 1). Analysis of clearance data approached in this way revealed that the DR2-positive group retained its difference from the "other" comparison group ($P = 0.037$). In addition the MT1-positive/DR2-negative group and the B8/DR3 group were each distinguishable from the "other" comparison group ($P = 0.023$ and $P = 0.027$, respectively). In contrast, the eight patients characterized by DR3 were not statistically distinguishable from the "other" comparison group ($P = 0.082$).

Fc Receptor Binding. In vitro data on EoxA rosette formation and IgG aggregate binding were obtained on 17 individuals. Of these only two had the B8 and DR3 antigens. Six subjects were DR2-positive and eight were MT1-positive. No statistically distinguishable differences in terms of rosette formation ($27.9 \pm 10.5\%$ [mean \pm SD] vs. $24.2 \pm 7.0\%$) or maximum binding and association constant (6.1 ± 5.0 vs. $5.9 \pm 3.4 \times 10^4$ /cell; 2.7 ± 1.8 vs. $3.3 \pm 1.7 \times 10^8$ M⁻¹, respectively) could be determined for the DR2 or MT1 individuals when compared to others.

EoxA Phagocytosis. Vmax was determined by Lineweaver-Burke analysis of phagocytic rate determined at various substrate concentrations (Table I). Vmax was significantly lower in the DR2 group when compared with all others ($P < 0.025$, Mann-Whitney U test, one-tailed). Similarly, subjects who were either MT1-positive or DR2-positive demonstrated significantly lower Vmax compared with others ($P < 0.01$). Since the B8/DR3 subgroup and MT1-positive/DR2-negative subgroup contained only two and three subjects, respectively, their difference compared with others could not be tested.

Discussion

A genetic predisposition to autoimmune diseases has been suggested by the association of various HLA antigens with diseases marked by altered immunoreactivity. Several studies indicate that clinically normal individuals with a particular HLA antigen may manifest some differences in immune function usually thought to be a feature of the particular disease associated with that antigen (12–15). MPS-clearance dysfunction is most consistent and most profound in SLE (4, 5). Lawley et al. (6)

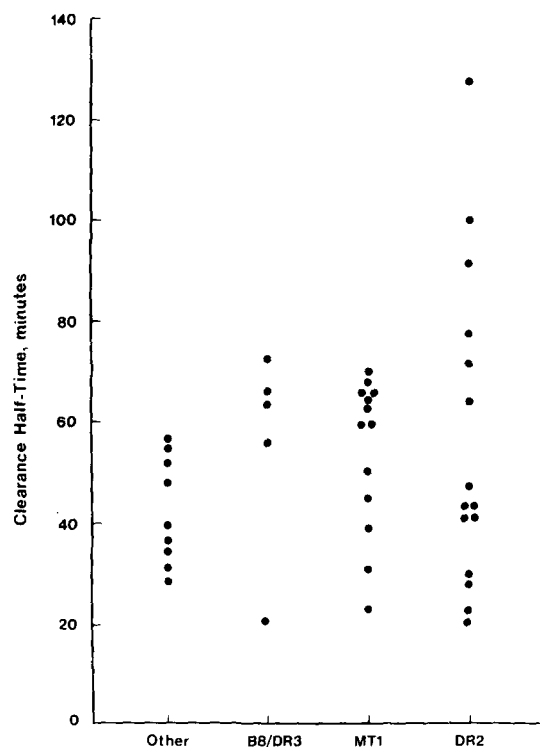


FIG. 1. MPS-clearance half-times in disease-free individuals by immunogenetic subgroup. Compared to upper 95% confidence limit of the control "other" group, HLA-B8/DR3 individuals ($P = 0.027$), MT1-positive/DR2-negative individuals ($P = 0.023$) and DR2-positive individuals ($P = 0.037$) each contain more individuals than expected with abnormally long clearance times. The DR2 group is significantly more dispersed than both the non-DR2 groups, B8/DR3 and MT1 ($P < 0.01$; Mood's test of dispersion), and the "other" subgroup ($P < 0.04$).

TABLE I
Phagocytosis of Antibody-sensitized Bovine Erythrocytes by HLA-DR2 Subjects

HLA-DR	Lineweaver-Burke analysis*			Phagocytic rate‡	
	Vmax	K_m	Slope	10:1	50:1
DR2 (n = 6)	2.1 ± 0.7 §	6.8 ± 2.9	4.2 ± 3.0	1.4 ± 0.4	1.9 ± 0.6
Non-DR2 (n = 11)	3.9 ± 2.0	9.4 ± 5.6	2.7 ± 0.8	2.0 ± 0.7	3.4 ± 1.6
P	$P < 0.025$	NS	$0.05 < P < 0.10$	< 0.025	< 0.025

* Determined by least squares linear regression of the double reciprocal representation of phagocytic rate at different substrate concentrations. Vmax, maximum velocity in erythrocytes per monocyte per hour; K_m , Michaelis-Menten constant in erythrocytes per monocyte; slope, slope in h^{-1} .

‡ Specific phagocytic rates (erythrocytes per monocyte per hour) at two different ratios of erythrocytes to monocytes.

§ Mean \pm SD.

|| $P > 0.10$.

recently reported that normals with the HLA-B8/DR3 phenotype had delayed Fc receptor-mediated clearance of IgG-sensitized erythrocytes. Since SLE has been associated with increased frequencies of DR2, DR3, and MT1 (1-3), we sought to evaluate MPS clearance in normal individuals with these specific HLA antigens.

Our results indicate that in young, healthy Caucasian adults, delayed MPS clearance is evident in those with either DR2, MT1 (whether DR2+ or DR2-), or B8/DR3. Among our subjects, DR3 alone did not identify a group with impaired

clearance. The central tendencies or means of these subgroups were not different. Analysis of the dispersion in the groups demonstrated that the distribution of values, especially in the DR2 individuals, was significantly larger. This finding implies that the DR2 group itself is heterogeneous with regard to MPS clearance and that the association of MPS dysfunction with DR2, although perceptible, is not absolute.

Fries and associates (16) investigated the saturable binding of monomeric IgG1 to Fc receptors in B8/DR3 individuals to see if altered binding might explain the Fc receptor-mediated clearance defect previously described. Just as with our subgroups, no differences between HLA-B8/DR3 individuals and others could be found with regard to Fc receptor binding.

Using the Fc receptor-dependent phagocytosis of EoxA, we have demonstrated altered phagocytosis in the DR2 group. Since rosette formation with an identically prepared EoxA was similar, differences in events other than or subsequent to ligand-receptor interaction may underlie the decrease in phagocytosis by blood monocytes in the DR2 group; the altered MPS clearance, however, depends on fixed mononuclear phagocytes that might reflect different properties than blood monocytes. Since our assay of phagocytosis involves a mixed mononuclear cell preparation, we cannot exclude the possibility that the difference in phagocytosis rests in the elaboration of a lymphokine-affecting monocyte function rather than in an intrinsic monocyte defect.

Intrinsic MPS dysfunction may contribute significantly to pathogenesis in SLE. For example, patients with nephritis have more pronounced MPS defects (5). The magnitude of MPS dysfunction is substantially larger in these patients than in our normals and has a large dynamic component, perhaps secondary to Fc receptor blockade, which changes with clinical status (17). Individuals with an immunogenetically determined difference in monocyte function might be more susceptible to such an additional effect. Altered MPS function may also signify more than altered handling of immune complexes and may indicate changes in other monocyte functions that involve specific receptor-ligand interactions and phagocytosis either for disposal or for further processing and presentation.

Summary

Normal individuals with an HLA haplotype containing either DR2, MT1, or B8/DR3 are more likely to have abnormally prolonged Fc receptor-mediated mononuclear phagocyte system (MPS) clearance of IgG-sensitized autologous erythrocytes than their normal counterparts without such haplotypes. Although measurement of Fc receptor binding by rosette formation and saturable IgG aggregate binding revealed no differences among groups, Fc receptor-mediated phagocytosis of IgG-sensitized bovine erythrocytes by monocytes was decreased in the DR2-positive and MT1-positive individuals. The basal *in vivo* MPS clearance in normal individuals may be immunogenetically determined and may reflect differences in phagocytic rates.

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References

1. Gibofsky, A., R. J. Winchester, M. Patarroyo, M. Fotino, and H. G. Kunkel. 1978. Disease associations of the Ia-like human alloantigens: contrasting patterns in rheumatoid arthritis and systemic lupus erythematosus. *J. Exp. Med.* **148**:1728.
2. Reinertsen, J. L., J. H. Klippel, A. H. Johnson, A. D. Steinberg, J. L. Decker, and D. L. Mann. 1978. B-lymphocyte alloantigens associated with systemic lupus erythematosus. *N. Engl. J. Med.* **299**:515.
3. Ahearn, J. M., T. T. Provost, C. A. Dorsch, M. B. Stevens, W. B. Bias, and F. C. Arnett. 1982. Interrelationships of HLA-DR, MB, and MT phenotypes, autoantibody expression, and clinical features in systemic lupus erythematosus. *Arthritis Rheum.* **25**:1031.
4. Frank, M. M., M. I. Hamburger, T. J. Lawley, R. P. Kimberly, and P. H. Plotz. 1979. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. *N. Engl. J. Med.* **300**:518.
5. Parris, T. M., R. P. Kimberly, R. D. Inman, J. S. McDougal, A. Gibofsky, and C. L. Christian. 1982. Defective Fc receptor-mediated function of the mononuclear phagocyte system in lupus nephritis. *Ann. Intern. Med.* **97**:526.
6. Lawley, T. J., R. P. Hall, A. S. Fauci, S. I. Katz, M. I. Hamburger, and M. M. Frank. 1981. Defective Fc-receptor functions associated with the HLA-B8/DRw3 haplotype. *N. Engl. J. Med.* **304**:185.
7. Kumagai, K., K. Itoh, S. Hinuma, and M. Tada. 1979. Pretreatment of plastic petri dishes with fetal calf serum. A simple method for macrophage isolation. *J. Immunol. Methods.* **29**:17.
8. Segal, D., and E. Hurwitz. 1977. Binding of affinity cross linked oligomers of IgG to cells bearing Fc receptors. *J. Immunol.* **118**:1338.
9. Bevington, P. R. 1969. Data Reduction and Error Analysis for Physical Sciences. McGraw-Hill, Inc., New York.
10. Kavet, R. I., and J. D. Brain. 1977. Phagocytosis: quantification of rates and intercellular heterogeneity. *J. Appl. Physiol.* **42**:432.
11. Daniel, W. W. 1978. Applied Non-parametric Statistics. Houghton Mifflin Co., Boston.
12. Solinger, A. M., R. Bhatnagar, and J. D. Stobo. 1981. Cellular, molecular, and genetic characteristics of T cell reactivity to collagen in man. *Proc. Natl. Acad. Sci. USA.* **78**:3877.
13. McCombs, C. C., and J. P. Michalski. 1982. Lymphocyte abnormality associated with HLA-B8 in healthy young adults. *J. Exp. Med.* **156**:936.
14. Cunningham-Rundles, S., C. Cunningham-Rundles, M. S. Pollack, R. A. Good, and B. Dupont. 1978. Response to wheat antigen in in vitro lymphocyte transformation among HLA B8 positive normal donors. *Transplant. Proc.* **10**:977.
15. Ambinder, J. M., N. Chiorazzi, A. Gibofsky, M. Fotino, and H. G. Kunkel. 1982. Special characteristics of cellular immune function in normal individuals of the HLA DR3 type. *Clin. Immunol. Immunopathol.* **23**:269.
16. Fries, L. F., R. P. Hall, T. J. Lawley, G. R. Crabtree, and M. M. Frank. 1982. Monocyte receptors for the Fc portion of IgG studied with monomeric IgG1: normal in vitro expression of Fc receptors in HLA B8/DRw3 subjects with defective Fc-mediated in vivo clearance. *J. Immunol.* **129**:1041.
17. Hamburger, M. I., T. J. Lawley, R. P. Kimberly, P. H. Plotz, and M. M. Frank. 1982. A serial study of splenic reticuloendothelial system Fc receptor functional activity in systemic lupus erythematosus. *Arthritis Rheum.* **25**:48.