Factors Influencing Reticulophagocytic Function in Insulin-treated Diabetes

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SUMMARY

The splenic component of reticulophagocytic function (RPF) was examined in 29 insulin-treated diabetic subjects (13 type I and 16 type II) by measurement of clearance of altered, radiolabeled, autologous erythrocytes. Double-isotope studies were performed with cells altered by: (1) preincubation with N-ethylmaleimide (NEM) and (2) coating with IgG antibody to the Rhesus (Rh) D antigen, labeled with ^{99m}Tc and ^{51}Cr, respectively. HLA typing for the A, B, and DR loci was performed in those patients showing a defect in the clearance of IgG-coated cells. Values for half-life ($t_{\frac{1}{2}}$) were correlated with the incidence of diabetic complications, levels of HbA_1, and circulating immune complexes (CIC). Two patterns of abnormal clearance were observed: first, an isolated defect of IgG-coated cell clearance in 7 patients (3 had the HLA B8/DR3 haplotype) and second, abnormal removal of both types of cell in a further 7 patients (3 had B8/DR3). There was no correlation between half-lives as measured by the two methods, although exclusion of the patients with a defect of IgG-coated cell clearance alone yielded a highly significant correlation for the remaining 15 Rh-positive patients ($P < 0.01$). Abnormalities of IgG-coated cell clearance were more frequent in patients with $HbA_1 > 9\%$ ($P < 0.02$), while $t_{\frac{1}{2}}$ of NEM-altered cells was significantly greater in patients with CIC ($P < 0.05$). There was no correlation between $t_{\frac{1}{2}}$ and the incidence of peripheral complications.

The influence of splenic blood flow (as measured by organ weight and retention of injected radiolabeled microspheres) on the $t_{\frac{1}{2}}$ of NEM-altered cells was examined in rabbits. The $t_{\frac{1}{2}}$ correlated with both splenic weight ($P < 0.001$) and retention of microspheres ($P < 0.001$).

Our data emphasize the value of double-isotope studies in assessing RPF in vivo. The isolated abnormality of IgG-coated cell clearance probably resulted from a specific defect of the splenic macrophage receptor and the B8/DR3 haplotype, and blood sugar control appeared to influence this abnormality. In addition to these factors, our animal studies suggested that splenic blood flow could contribute to the dual defect seen in a further 7 patients. These factors could hinder the removal of potentially tissue damaging macromolecules from the circulation of patients with diabetes. DIABETES 1984; 33:813–18.

Immunologic mechanisms are suspected to play a role in the pathogenesis of the microvascular complications of diabetes mellitus. Such a possibility is supported by the similarity between diabetic vascular lesions in the kidney and those seen in other immunologic renal diseases,[1] the finding of immunoreactive proteins in retinal and glomerular capillaries[2,3] and, more recently, the detection of circulating immune complexes (CIC) in a high percentage of both type I and type II diabetic patients.[4–6] Complexes have been found most frequently in patients with retinopathy[7] and their presence has been shown to correlate with the impaired clearance of radiolabeled microaggregated albumin.[8] Some authors have found both insulin and insulin antibody in vessel walls and have postulated that the accompanying lesions result from the deposition of insulin/anti-insulin complexes.[2,9] Other workers have not been able to confirm the deposition of these proteins.[10]

The clarification of factors responsible for the increased CIC in diabetes is important, as these complexes may play a role in the pathogenesis of microangiopathy. Essentially, increased levels can result from enhanced formation of complexes or reduced clearance by various components of the reticulophagocytic system (RPS). Frank and co-workers[11] have examined the latter aspect using radiolabeled, IgG-sensitized erythrocytes to test the functional capacity of the splenic macrophage IgG-Fc receptor. They showed the clearance of these cells was impaired significantly in patients with active systemic lupus erythematosus (SLE), and sug-

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gested that this caused persistence of immune complexes in the circulation, thus increasing their tissue-damaging potential. Subsequently, these authors have shown similar clearance defects in patients with dermatitis herpetiformis and in an increased percentage of healthy subjects with the B8/DR3 haplotype.[12] We have used the measurement of clearance of altered autologous erythrocytes to examine splenic macrophage function in 29 insulin-treated diabetic patients, with and without complications, and with varying degrees of blood sugar control. Double-isotope studies were used to simultaneously examine the clearance of IgG-coated red cells (to test splenic Fc receptors) and cells altered with N-ethylmaleimide (NEM) to examine splenic blood flow (and possibly non-Fc receptors). Studies with NEM-damaged cells were also performed in experimental animals and half-lives ($t_{\frac{1}{2}}$) correlated with splenic blood flow measured with isotope-labeled microspheres. HLA loci A, B, and DR were determined in those patients showing a major abnormality of clearance of IgG-coated cells to determine whether this was associated with the B8/DR3 haplotype. Correlations between clearance times for both types of altered cell, levels of CIC, and the presence of complications were also examined. The basis for defects in the clearance of autologous cells was further investigated in vitro using human peritoneal macrophages and various indicator substances in the presence or absence of diabetic sera.

MATERIALS AND METHODS

Patients. Twenty-nine patients with insulin-treated diabetes mellitus (13 type I and 16 type II) were studied. Clinical details are shown in Table 1. The age range was 18–68 yr (mean: 45 yr) and the duration of diagnosed diabetes varied from 2 wk to 46 yr (mean: 11 yr). Fourteen patients had retinopathy and/or nephropathy and six were receiving antihypertensive therapy. Diabetic retinopathy was assessed by an ophthalmologist with the assistance of fluorescein angiography; patients were divided into one of three groups: (1) no retinal changes, (2) background retinopathy, and (3) proliferative retinopathy. Diabetic nephropathy was diagnosed in 6 patients with ≥ 400 mg protein in a 24-h urine collection and with or without impairment of creatinine clearance. There were no serologic features (such as positive antinuclear factors or complement abnormalities) to suggest another cause for glomerular damage. Three of the 6 patients with significant proteinuria underwent renal biopsy, which confirmed typical glomerulosclerotic changes. Diabetes control at the time of the red cell clearance study was assessed by the measurement of glycosylated hemoglobin A_1 (HbA_1) (Bio-Rad column assay) and blood glucose (Ames Glucometer). All patients had serum creatinine, serum albumin, 24-h urinary protein excretion, and creatinine clearance measured on the day of the study using standard techniques. No patient was nephrotic and serum albumin estimations ranged from 32 to 45 g/L (mean 37 g/L). The serum creatinine was elevated (0.12–0.21 mmol/L) in 5 patients (see Table 1).

Complement and immune complex assays. C1q, C4, and C3 were measured by radial immunodiffusion using monospecific antisera (Dako, Flow Lab).[13] Two different assays were used to measure CIC: (1) the C1q binding assay (C1q.BA) described by Zubler and co-workers[14] and (2) a modified Raji cell radioimmunoassay (RIA) using monospecific antisera to IgG, IgM, and IgA.[15,16] Results of the Raji cell RIA were expressed as percent pooled normal human serum (NHS). The upper limit of normal (mean + 2 SD) for each assay, as determined on sera from 30 healthy donors, was C1q.BA—16% precipitable ^{125}I-C1q; and Raji cell RIA—IgG: 122%; IgM: 161%; and IgA: 150%. Co-efficients of intraassay variation were: C1q.BA: 4.8%; and Raji cell RIA—IgG: 1.9%; IgM: 3.4%; and IgA: 5.3%. Co-efficients of interassay variations were: C1q.BA: 9.0% and Raji cell RIA—IgG: 8.4%; IgM: 7.4%; and IgA: 9.1%. All immune complex assays were performed by one member of the laboratory staff. Raji cells were tested for viability by trypan blue exclusion and for adequate complement surface receptors using complement-reacted sensitized sheep erythrocytes (EAC).

Tissue typing. HLA A, B, and DR typing was performed by the Tissue Typing Laboratory of the N.S.W. Red Cross Blood Transfusion Service using standard microcytotoxicity tests with antisera standardized at the VIth and VIIth International Histocompatibility Workshops.

Radiolabeling of erythrocytes. Red cells were radiolabeled with either sodium pertechnetate (^{99m}Tc) or sodium chromate (^{51}Cr). (1) ^{99m}Tc: Sterile stannous gluconate solution (Australian Atomic Energy Commission) 0.2 ml (equivalent to 2 μg tin) was added to 6 ml heparinized venous blood in a sterile vacutainer and the tube gently rotated at room temperature for 5 min. The sample was then centrifuged in an inverted position at 1500 rpm for 5 min and 2 ml packed cells removed, taking care not to disturb the supernatant. The packed red cells were then added to a second sterile tube containing 2 ml saline (Lancet Pharmaceuticals Ltd., Australia) and 15 mega-Becquerel (MBq) of ^{99m}Tc (1 MBq = 27 μCi), incubated at room temperature for a further 5 min, again centrifuged at 1500 rpm for 5 min, and the supernatant removed. Any evidence of hemolysis was noted. Labeling efficiency was calculated to be greater than 95%. (2) ^{51}Cr: Venous blood (10 ml) was labeled with 2 MBq ^{51}Cr using the method published by Frank et al.[11] Labeling efficiency was greater than 80%.

Alteration of radiolabeled erythrocytes. Radiolabeled red cells were altered by one of two methods: (1) Chemical alteration of the red cell membrane. The erythrocytes were incubated for 1 h at room temperature with the sulfhydryl inhibitor, NEM (Calbiochem-Behring) in a dose of 20 μmol/ml packed red cells.[17] (2) Coating with antibody. The red cells were coated with a noncomplement-fixing IgG antibody to the Rhesus-D antigen (Commonwealth Serum Laboratories, Australia) by incubating the red cell/antibody mixture for 30 min at 37°C.[18] The packed red cells were washed at least once in sterile saline after the damaging procedure and resuspended in a final volume of 5–6 ml sterile saline for reinjection. No significant elution of the radioisotope occurred during the damaging process. The number of molecules of IgG anti-D antibody/cell was 4195 ± 363 (mean ± SD) for control subjects and 4191 ± 330 for diabetic patients.

Clearance studies. Ethics Committee approval and the informed consent of each subject were obtained before the study. The protocol was that outlined by Lockwood et al.[19] Briefly, the altered radiolabeled erythrocytes were injected

TABLE 1
Clinical details of patients

Patient	Sex/Age (yr)	Diabetes Duration (yr)	Diabetes Type	HbA_1 (%)	Retinopathy	Proteinuria (mg/24 h)*	Serum creatinine (mmol/L)†	Immune complexes C1q.BA	Immune complexes Raji RIA
1	F/25	10	I	12.5	–	–	0.08	–	–
2	M/47	1 mo	II	16.0	–	–	0.11	–	–
3	F/58	14	II	12.3	–	–	0.08	–	–
4	F/25	9	I	9.2	BG	–	0.08	+	+
5	M/64	12	II	13.5	BG	1560	0.12	–	–
6	M/44	5	II	8.7	BG	–	0.08	–	+
7	M/59	7	II	8.3	BG	600‡	0.21	+	–
8	M/58	20	II	7.5	BG	1300‡	0.18	–	–
9	M/59	46	I	8.4	–	710	0.14	–	+
10	F/60	40	I	11.7	BG	–	0.08	–	+
11	F/19	17	I	11.0	PR	–	0.07	–	–
12	M/40	2 wk	II	12.2	–	–	0.07	–	+
13	M/31	1 mo	I	16.3	–	–	0.08	–	–
14	F/53	1 mo	II	17.5	BG	–	0.07	+	+
15	M/68	20	II	6.8	–	–	0.09	–	+
16	F/27	1.5	I	10.1	–	–	0.09	+	+
17	F/48	2	II	18.1	–	–	0.09	–	–
18	F/33	7	I	8.9	–	–	0.09	–	+
19	F/57	27	II	11.2	PR	–	0.06	–	–
20	F/23	12	I	11.1	–	–	0.07	–	+
21	M/42	9	I	14.3	–	–	0.11	–	+
22	F/43	1.5	II	12.9	–	–	0.10	–	–
23	F/55	25	II	8.3	BG	–	0.10	–	–
24	M/50	9	II	9.7	BG	400‡	0.11	+	–
25	M/54	10	II	6.9	PR	1780	0.21	–	+
26	F/52	17	I	11.9	PR	–	0.07	–	–
27	M/43	12	I	18.3	–	–	0.09	–	–
28	M/54	1 mo	II	16.4	–	–	0.08	+	–
29	F/18	5	I	12.8	–	–	0.07	–	+

BG: background retinopathy; and PR: proliferative retinopathy.
*Normal ≤ 150 mg/24 h, †normal = 0.06–0.11 mmol/L, ‡renal biopsy performed; and – = not detected or within normal range.

over 1 min. Three minutes after the midpoint of the injection, a 5-ml sample of venous blood was removed and similar samples collected at 5-min intervals for a further 25 min. One-milliliter aliquots of the timed whole-blood specimens were counted in a well-type gamma counter (LKB Wallace 1239 Ultrogamma) and the $t_{\frac{1}{2}}$ calculated from the whole-blood radioactivity disappearance curve. The specimen collected at 3 min was used as the 100% reference value to avoid errors caused by recirculation of isotope during the first 1–2 min after injection (see Mollison[20]).

In 4 control subjects (including 1 Rh-negative individual and 1 previously splenectomized subject), the site of clearance of NEM-altered cells (tested in 1 Rh-positive control) and IgG-coated cells was determined by radionuclide scanning. This showed specific localization of isotope to the spleen (in the 2 Rh-positive subjects) with no detectable uptake in the liver. There was no significant clearance of cells in the Rh-negative and splenectomized subjects. A similar finding has been reported by Lockwood and coworkers[19] using the same protocol for the preparation of cells and measurement of turnover.

Double-isotope studies. In all Rh-positive patients, the clearance of NEM-altered cells (labeled with ^{99m}Tc) was compared with that of IgG-coated cells (labeled with ^{51}Cr) by the use of a double-isotope technique. The separately prepared cells were injected simultaneously and specimens collected as outlined above. The samples were counted for ^{99m}Tc content within 1 h of the clearance study and then recounted after 24 h (after decay of the ^{99m}Tc) to determine the amount of ^{51}Cr present in each of the timed samples.

In vitro macrophage studies. Peritoneal macrophages were harvested from patients on uncomplicated maintenance peritoneal dialysis. Dialysis fluid was centrifuged at 1000 rpm for 10 min and the cells washed 3 times in cold RPMI 1640/20% decomplemented fetal calf serum (FCS) (CSL, Australia). The pellet was resuspended in fresh cold medium (RPMI/20% FCS) and about 5×10^5 cells seeded onto flat-bottomed microtiter wells (Nunc, Denmark). After 1 h incubation at 37°C, the nonadherent cells were removed by washing and the adherent macrophages examined for viability by the trypan blue exclusion test. The adherent macrophages were then incubated for a further 1 h at 37°C with fresh medium containing 20 μl of various test substances and/or 200 μl of antibody-coated erythrocytes (EA). The test reagents were IgG-coated Latex particles (Calbiochem-Behring), carbon particles (Norit-A), pooled normal human serum (NHS), and sera from patients with diabetes. After the second incubation period, the cells were again washed, fixed, stained, and examined by light microscopy. The ability of these cells to bind erythrocytes was assessed in the presence of medium alone, serum, inert particulate matter, or Fc-bearing material (i.e., IgG-coated Latex particles). Three low-power fields (magnification × 200) were examined and the percentage of macrophages forming EA rosettes calculated.

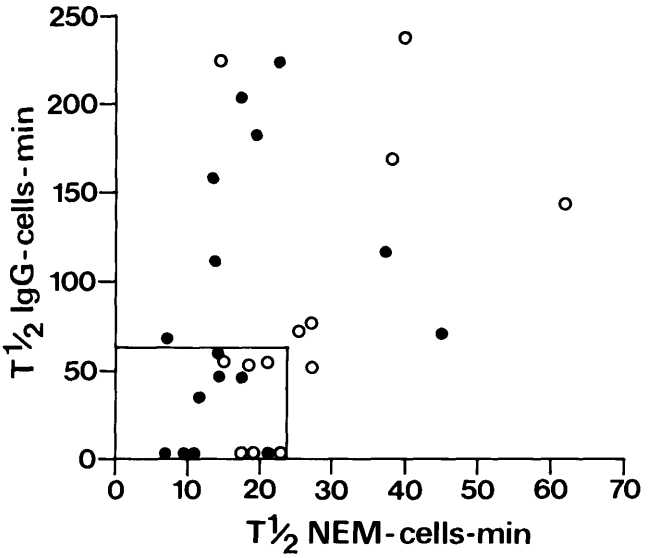

FIGURE 1. Comparison of values for $t_{\frac{1}{2}}$ of NEM- and IgG-altered autologous red cells. The upper limit of normal (mean + 2 SD) for each method is shown. ○, Type I diabetes; ●, type II diabetes. The values on the x-axis represent Rh-negative patients.

Results were expressed as the mean of at least 3 separate experiments performed in duplicate.

Clearance studies in animals. A colony of specific-pathogen-free rabbits was bred, and litter mates weighing between 2.5 and 3.5 kg were used in all the experiments. The animals were kept in separate cages and fed on a standard laboratory diet. All animals had aortic and central venous catheters inserted under general anesthesia (i.v. phenobarbitone) on the day before study. Two groups of experiments were then performed to examine the correlation between $t_{\frac{1}{2}}$ NEM-altered cells and splenic blood flow under both normal conditions and those following injection of an immune complex load. In group I (3 rabbits), clearance studies were carried out using NEM-altered, ^{51}Cr-labeled autologous cells injected into the venous catheter; the turnover protocol has been previously described.[21] At the end of the study, 1 ml ^{99m}Tc-labeled human serum albumin (HSA) microspheres (3M Company, Minnesota) was injected into the aortic catheter. The animals were killed 1 min later by the i.v. injection of potassium chloride (to assure immediate cardioplegia) and phenobarbitone. Organs were removed, weighed, and counted for residual ^{99m}Tc. The assessment of splenic blood flow was based on organ weight and percent injected ^{99m}Tc trapped in the spleen. In group II (4 rabbits), clearance studies with NEM-altered cells were performed as for group I. One hour after the red cell clearance, each animal was injected with soluble complexes of bovine serum albumin (BSA)/anti-BSA (prepared in 10-fold excess by a standard technique) via an ear vein. Fifteen minutes later, a second clearance was undertaken to examine the effect of IC on the turnover of NEM-altered cells and, in particular, on splenic blood flow. At the end of this second study, the animals were injected with microspheres (as in group I), killed, and organs weighed and counted for residual ^{99m}Tc. Data were not used from those animals that showed evidence of cardiorespiratory distress during the course of the experiments.

Statistics. Linear regression analysis, with calculation of the coefficient r, was used to examine the correlation between (1) $t_{\frac{1}{2}}$ for NEM- and IgG-treated cells, (2) NEM clearance ($t_{\frac{1}{2}}$) and splenic weight or ^{99m}Tc content, and (3) $t_{\frac{1}{2}}$ for each method and levels of CIC and HbA_1.

The correlation between the presence of each peripheral complication and abnormalities of red cell clearance was tested by Chi-square (X^2) analysis and by Students *t* test (using individual values for $t_{\frac{1}{2}}$).

RESULTS

Red cell clearance studies. (1) Control subjects: the $t_{\frac{1}{2}}$ clearance of NEM-treated erythrocytes in 10 normal subjects was 15.8 ± 8.0 min (mean ± 2 SD) while the clearance time of antibody-coated cells was 57.6 ± 5.2 min in 8 control studies. (2) Patients: $t_{\frac{1}{2}}$ clearance times for both methods are shown in Figure 1. Seven patients (5 type I and 2 type II) had abnormal $t_{\frac{1}{2}}$ for both NEM- and IgG-altered cells, while 7 (1 type I and 6 type II) had impaired clearance of only IgG-coated cells. A further 7 patients were Rh-negative and were not studied with IgG-coated cells. One subject showed an isolated defect of NEM-altered cell clearance but this abnormality was mild ($t_{\frac{1}{2}}$: 27 min). There was no correlation between $t_{\frac{1}{2}}$ NEM-altered cells and $t_{\frac{1}{2}}$ IgG-coated cells; however, exclusion of the 7 subjects with an isolated IgG-coated cell clearance defect (they were considered likely to represent a separate functional group) yielded a highly significant correlation in the remaining 15 Rh-positive patients ($r = 0.68$, $P < 0.01$).

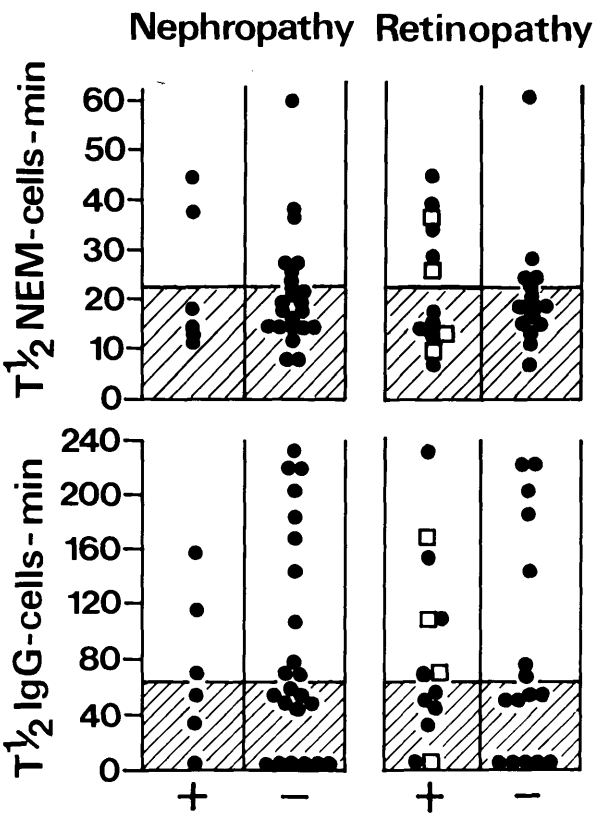

FIGURE 2. $T_{\frac{1}{2}}$ for NEM- and IgG-altered cells in patients with (+) and without (−) nephropathy and retinopathy. The upper limit of normal for each method is shown. □, Patients with proliferative retinopathy. No significant differences were found.

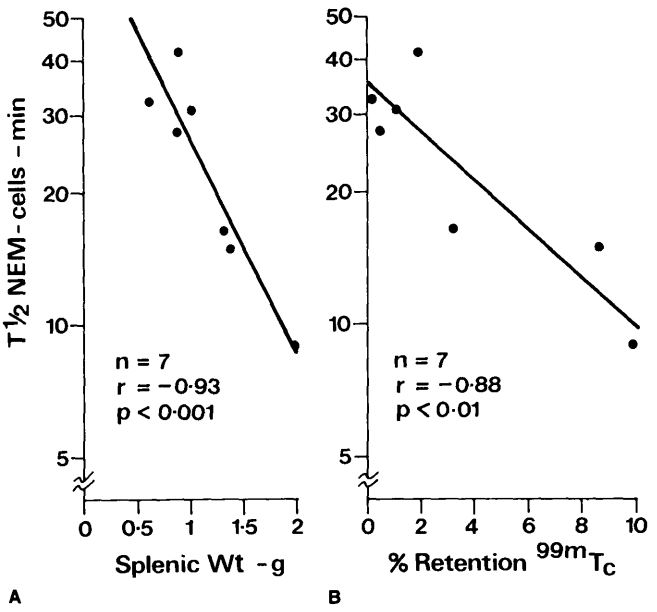

FIGURE 3. Data from rabbit experiments showing correlations between $t_{\frac{1}{2}}$ NEM-altered cells and (A) splenic weight and (B) %^{99m}Tc retained in spleen. There was a highly significant correlation in each case ($P < 0.001$ and $P < 0.01$, respectively). The clearance studies were performed under normal conditions in 3 rabbits and after the injection of preformed immune complexes in the other 4 animals.

Abnormalities of clearance of IgG-coated cells were more frequent in patients with $HbA_1 > 9\%$ (X^2 analysis; $P < 0.02$) and values for $t_{\frac{1}{2}}$ were also significantly higher in this group ($t = 2.17$; $P < 0.05$). No relationship was found between NEM-altered cell clearance and HbA_1.

The $t_{\frac{1}{2}}$ NEM-altered cells was significantly greater in patients with CIC (detected by either method) than in those without CIC ($t = 2.36$; $P < 0.05$); there was no corresponding difference in the $t_{\frac{1}{2}}$ of IgG-coated cells. There was no significant correlation between $t_{\frac{1}{2}}$ of NEM- or IgG-altered cells and levels of CIC measured by the C1q.BA or the Raji cell RIA. A comparison of red cell clearance times for patients with and without retinopathy and/or nephropathy are shown in Figure 2. Values for $t_{\frac{1}{2}}$ (measured by either method) were comparable in patients with and without complications (Students *t* test). The frequency of abnormalities of clearance (for either cell type) in patients with complications was no different from that in patients without complications (X^2 analysis with Yates' correction).

The separate analysis of patients with type I and type II diabetes showed no correlation between values for $t_{\frac{1}{2}}$, as measured by the two methods. Values for $t_{\frac{1}{2}}$ of NEM-altered cells were higher in type I patients with retinopathy than in those without this complication ($t = 2.84$; $P < 0.02$) but this correlation did not apply to nephropathy or to studies performed with IgG-altered cells.

Studies of complement and immune complexes. Levels of C1q were reduced in 6 patients, while C4 and C3 were consistently normal. Six patients had detectable CIC by the C1q.BA and 13 were positive for one or more Ig classes with the Raji cell RIA (IgG: 8 positive, IgM: 2 positive, and IgA: 9 positive). Sixteen patients had CIC detected by one or the other assay.

HLA typing. This was performed in 13 of the 14 patients with abnormal clearance of IgG-coated cells (with or without an associated defect of NEM-altered cell turnover). The B8/DR3 haplotype was present in 3 of 6 patients with an isolated defect of IgG-coated cell clearance (the seventh patient was not available for typing). None of these 3 patients had detectable CIC, while complexes were found in 1 of the remaining 3 B8/DR3-negative patients. Three of the 7 patients with abnormal clearance of both cell types were positive for B8/DR3; CIC were detected in 2 of these and in each of the 4 B8/DR3-negative patients with a dual clearance defect.

Effect of diabetic sera on phagocytosis. The phagocytic function of human peritoneal macrophages was not inhibited by serum from any of the patients. Both inert material and antibody-coated particles were bound at the same rate as in control experiments.

Clearance studies in animals. Correlations between $t_{\frac{1}{2}}$ NEM-altered cells and splenic weight or splenic uptake of ^{99m}Tc-labeled microspheres are shown in Figures 3A and 3B, respectively. There was a highly significant inverse correlation between $t_{\frac{1}{2}}$ and splenic weight ($r = 0.93$; $P < 0.001$) and $t_{\frac{1}{2}}$ and uptake of ^{99m}Tc ($r = 0.88$; $P < 0.01$). In group II animals, the preliminary NEM-altered cell clearance times were similar to those of the group I control animals. The subsequent injection of BSA/anti-BSA complexes caused a mean increase in $t_{\frac{1}{2}}$ of 191% (range: 58–323%) above the first clearance value.

DISCUSSION

The splenic clearance of NEM- and IgG-altered erythrocytes was significantly impaired in patients with diabetes compared with healthy control subjects ($P < 0.05$ and $P < 0.001$, respectively). Two patterns of clearance abnormality were observed. Seven patients, including 6 with type II diabetes, showed a defect in the removal of IgG-coated cells alone and this probably reflects functional impairment of the splenic IgG-Fc receptor. Several factors observed in these patients could have contributed to this abnormality. First, approximately half of the group had the B8/DR3 haplotype, which is associated with similar clearance defects in approximately 50% of healthy individuals.[12] Second, abnormalities of IgG-coated cell clearance were more prevalent in patients with $HbA_1 > 9\%$; the mean level of HbA_1 in the 7 subjects with an isolated IgG-coated cell defect was 14.4% compared with 10.5% in patients with normal $t_{\frac{1}{2}}$. Finally, 1 patient had detectable CIC, but these were not found with the IgG-specific Raji RIA and it is uncertain whether they influenced the clearance time. Although receptor abnormalities might be expected to be more frequent in older patients, we wish to emphasize that the mean age of this group was lower than that with normal IgG-coated cell clearance.

Our studies in experimental animals suggest that the dual clearance abnormality seen in a further 7 patients could reflect, to some extent, reduction in splenic blood flow. These animal studies showed a striking inverse correlation between $t_{\frac{1}{2}}$ NEM-altered cells and blood flow, as measured by either organ weight ($P < 0.001$) or retention of radiolabeled microspheres ($P < 0.01$). Furthermore, the $t_{\frac{1}{2}}$ of NEM-altered cells was significantly higher in type I patients with micro-

vascular disease (as defined by the presence of retinopathy). Three of this group also showed the B8/DR3 haplotype and 6 had detectable CIC at the time of study. The possibility that the clearance of NEM-altered cells is influenced by non-Fc receptors cannot be completely discounted. Such a proposal has been made for other nonspecifically altered material (such as heat-damaged red cells or microaggregated albumin). However our failure to find a significant number of patients with a defect of NEM-altered cell clearance alone makes this unlikely.

We were unable to show a correlation between the presence or level of CIC and the clearance of IgG-coated cells. This finding is different from that reported by Frank and co-workers[11] in studies of patients with active SLE in which they used the same techniques (i.e., C1q.BA and Raji RIA) to detect immune complexes. These authors also showed improvement in the clearance defect with control of disease activity[22] and Lockwood et al.[19] demonstrated that the receptor defect could be reversed with plasma exchange. Lawley and co-workers,[12] however, found no correlation between the detection of CIC, disease activity, and red cell clearance defects in patients with dermatitis herpetiformis. This disease, like type I diabetes, has a distinct association with the B8/DR3 haplotype. Similarly, studies with IgG-coated and heat-damaged cells in glomerulonephritis have shown impaired clearance of the former to correlate with the presence of B8/DR3 rather than the detection of CIC.[23] Our data resemble these latter two reports in that factors other than CIC seem likely to be important determinants of red cell clearance (in our group of patients). Such a proposal is supported by the failure to demonstrate serum inhibition of uptake of preformed particles onto the surface of peritoneal macrophages. It is recognized that these cells may differ substantially from those residing in the spleen, but our in vitro studies were confined to the examination of serum/cell-surface interaction rather than the intrinsic phagocytic properties of the cell itself. Human peritoneal macrophages have been shown to contain IgG-Fc receptors.[24]

Irrespective of the basis for the clearance defect observed in many of our patients, such abnormalities could interfere with the effective removal of circulating macromolecules with tissue-damaging potential. Although our patients did not exhibit the clearance characteristics of more established immune complex-mediated diseases, these defects could be relevant to the long-term complications of diabetes.

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REFERENCES

[1] Blumenthal, H. T., Berns, A. W., Owens, C. T., and Hirata Y.: The pathogenesis of diabetic glomerulosclerosis. I. The significance of various histopathological components of the disease. Diabetes 1962; 11:296–307.

[2] Werner, A. U., and Larsen, H.-W.: Immunohistological studies on human diabetic and non-diabetic eyes. I. Fluorescent labelling of insulin and insulin antibodies. Acta Ophthalmol. 1969; 47:937–55.

[3] Burkholder, P. M.: Immunohistopathologic study of localised plasma proteins and fixation of guinea-pig complement in renal lesions of diabetic glomerulosclerosis. Diabetes 1965; 14:755–70.

[4] Irvine, W. J., Al-Khateeb, S. F., Di Mario, U., Feek, C. M., Gray, R. S., Edmond, B., and Duncan, L. J.: Soluble immune complexes in the sera of newly diagnosed insulin-dependent diabetics and in treated diabetics. Clin. Exp. Immunol. 1977; 30:16–21.

[5] Kumar, D., and Quismorio, F. P.: Circulating immune complexes in diabetic patients. Abstract. Diabetes 1978; 27 (Suppl. 2):486.

[6] Charlesworth, J. A., Campbell, L. V., Catanzaro, R., Pussell, B. A., Pasterfield, G. V., and Peake, P.: Immune complexes in diabetes mellitus: studies of complement utilisation and tissue deposition. J. Clin. Lab. Immunol. 1982; 8:163–68.

[7] Irvine, W. J., Di Mario, U., Guy, K., Iavicoli, M., Pozzilli, P., Lumbroso, B., and Andreani, D.: Immune complexes and diabetic microangiopathy. J. Clin. Lab. Immunol. 1978; 1:187–91.

[8] Iavicoli, M., Di Mario, U., Pozzilli, P., Canalese, J., Ventriglia, L., Galfo, C., and Andreani, D.: Impaired phagocytic function and increased immune complexes in diabetics with severe microangiopathy. Diabetes 1982; 31:7–11.

[9] Mancini, A. M., Zampa, G. A., Geminiani, G. D., and Vecchi, A.: Experimental nodular "diabetic-like" glomerulosclerosis in guinea pigs following long-acting, heterologous insulin immunisation. Diabetologia 1969; 5:155–66.

[10] Westberg, N. G., and Michael, A. F.: Immunohistopathology of diabetic glomerulosclerosis. Diabetes 1982; 21:163–74.

[11] Frank, M. M., Hamburger, M. I., Lawley, T. J., Kimberley, R. P., and Plotz, P. H.: Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. N. Engl. J. Med. 1979; 300:518–23.

[12] Lawley, T. J., Hall, R. P., Fauci, A. S., Katz, S. I., Hamburger, M. I., and Frank, M. M.: Defective Fc-receptor functions associated with the HLA-B8/DRW3 haplotype: studies in patients with dermatitis herpetiformis and normal subjects. N. Engl. J. Med. 1981; 304:185–92.

[13] Mancini, G., Carbonara, A. L., and Heremans, J. F.: Immunochemical quantitation of antigens by radial immunodiffusion. Immunochemistry 1965; 2:235–54.

[14] Zubler, R. H., Lange, G., Lambert, P. H., and Miescher, P. A.: Detection of immune complexes in unheated sera by a modified ^{125}I-C1q binding test. J. Immunol. 1976; 116:232–35.

[15] Theofilopoulos, A. N., Wilson, C. B., and Dixon, F. J.: The Raji cell radioimmune assay for detecting immune complexes in human sera. J. Clin. Invest. 1976; 57:169–82.

[16] Quin, J. W., Charlesworth, J. A., and Macdonald, G. J.: The detection of immune complexes of different immunoglobulin class. Aust. NZ J. Med. 1979; 9:644–49.

[17] Jacob, H. S., and Jandl, J. H.: Effects of sulfhydryl inhibition on red blood cells. II. Studies in vivo. J. Clin. Invest. 1962; 41:1514–23.

[18] Jaffe, C. J., Vierling, J. M., Jones, E. A., Lawley, T. J., and Frank, M. M.: Receptor specific clearance by the reticuloendothelial system in chronic liver disease. J. Clin. Invest. 1978; 61:1069–77.

[19] Lockwood, C. M., Worlledge, S., Nicholas, A., Cotton, C., and Peters, D. K.: Reversal of impaired splenic function in patients with nephritis or vasculitis (or both) by plasma exchange. N. Engl. J. Med. 1979; 300:524–30.

[20] Mollison, P. L.: Blood Transfusion in Clinical Medicine, 5th edit. Oxford, Blackwell Scientific Publications, 1972:466.

[21] Lawrence, S., Lockwood, C. M., and Peters, D. K.: Studies on NEM-treated erythrocyte clearance in the rabbit, with special reference to the effects of circulating immune complexes. Clin. Exp. Immunol. 1981; 44:433–39.

[22] Hamburger, M. I., Lawley, T. J., Kimberley, R. P., Plotz, P. H., and Frank, M. M.: A serial study of splenic reticuloendothelial system Fc receptor functional activity in systemic lupus erythematosus. Arthritis Rheum. 1982; 25:48–54.

[23] McGinley, E., Henderson, N., Martin, W., and Boulton-Jones, M.: Defective reticuloendothelial Fc-receptor specific function associated with the HLA B8-DR3 haplotype in idiopathic membranous glomerulonephritis. Kidney Int. 1982; 21:668.

[24] Dorrington, K. J.: Properties of the Fc-receptor on macrophages. Immunol. Commun. 1976; 5:263–80.