Tissue Antigens (1983) 21, 251–256

All monocyte antigens are not expressed on renal endothelium

W. M. BALDWIN III[1], F. H. J. CLAAS[2], L. C. PAUL[1], T. A. SPRINGER[3], G. F. J. HENDRIKS[2], L. A. VAN ES[1] and J. J. VAN ROOD[2]

[1]Department of Nephrology and [2]Department of Immunohematology, University Hospital, Leiden, The Netherlands; [3]Department of Pathology, Harvard Medical School, Boston, Massachusetts

Recently a tissue restricted antigen system, which is expressed on endothelial cells and monocytes (E–M antigens) but not lymphocytes, has been associated with kidney graft rejection. In screening sera from recipients of kidney, bone marrow or skin grafts for possible reactivity with endothelial cell antigens, we have found that all (13 of 13) endothelial reactive sera also reacted with monocytes, but that many (21 of 34) monocyte reactive sera did not react with endothelial cells. Additionally, one well-defined monoclonal antibody (M1 70), which was cytotoxic for human monocytes, neither stained renal endothelium nor was absorbed by renal endothelium when perfused through a human kidney. Thus, not all monocyte antigens appear to be expressed in high concentrations on renal vascular endothelium. This may explain why monocyte reactive antibodies do not always correlate with kidney graft rejection.

Received for publication 5 July, revised, accepted 8 November 1982

Minor histocompatibility antigens and tissue specific antigens have been assumed to account for the 5–15% of HLA matched cadaver and living related transplants which are rejected (Opelz et al. 1977). Recently reported examples of human renal graft rejections related to male specific (H–Y) minor histocompatibility antigens (Pfeffer & Thorsby, 1982) and tissue restricted endothelial-monocyte (E–M) antigens (Paul et al. 1979) have increased interest in this concept.

E–M antigens are not detected by conventional tissue typing techniques because they are not expressed on lymphocytes, platelets or erythrocytes (Cerilli et al. 1981; Paul et al. 1979; Stastny, 1978; Thompson et al. 1980). The discovery by Moraes and Stastny (1977) that E–M antigens were shared by endothelial cells and monocytes raised hopes that E–M antigens could be conveniently detected by tissue typing monocytes. Indeed, all of the endothelial antigens reported to date have been shared by monocytes (Paul et al. 1979; Stastny, 1978; Thompson et al. 1980). However, in the course of screening sera for E–M antibodies, we have found several sera that react with monocyte specific antigens but not

Table 1.
Comparison of antibody reactivity with monocytes and renal peritubular capillary endothelial cells.

Sera Source	Panel of renal biopsies[c]			Panel of monocytes[d]		
	0%	1–50%	51–100%	0%	1–50%	51–100%
Kidney recipients I[a]	0	3	4	0	3	4
Kidney recipients II[b]	14	4	0	0	16	2
Skin recipients[b]	4	1	0	0	4	1
Bone marrow recipients[b]	1	0	1	0	1	1
Transfusion recipients[b]	2	0	0	0	0	2

a. First found positive on endothelial cells and then tested on monocytes.
b. First found positive on monocytes and then tested on endothelial cells.
c. Biopsies from 6–20 kidneys were stained with each sera; data are expressed as percent biopsies with positive staining of the peritubular capillary endothelium.
d. Monocytes from 6–10 donors were reacted with each sera; data are expressed as percent donors with positively reacting monocytes.

Table 2.
Sensitivity of endothelial cell and monocyte assays in detecting E–M activity in unabsorbed and absorbed serum.

Test	End Point	Titer of serum after absorption with		
		Erythrocytes[1]	Lymphocytes[1]	Monocytes[1]
Endothelial	Strong	32	16	0
Staining	Weak	128	32	1
Monocyte	100%	64	16	0
Cytotoxicity	50%	128	32	1

1. The serum was extensively absorbed with erythrocytes and subsequently with either splenic lymphocytes or monocytes from the donor of the kidney biopsy used to test for endothelial reactivity.

stain the peritubular capillary (PTC) endothelial cells of renal biopsies were cytotoxic for monocytes from 10–80% of ten cell donors and stained the PTC endothelium of 22–67% of a panel of eighteen renal biopsies (Table 1). In contrast, of eighteen sera from renal transplant recipients, which first were found to be cytotoxic for monocyte specific antigens, only four stained PTC endothelium.

Sera which were cytotoxic for monocytes but did not stain endothelial cells were not limited to renal transplant recipients, but also were obtained from nine patients who rejected skin or bone marrow transplants or had adverse reactions to transfusions (Table 1). Finally, a well-characterized rat monoclonal antibody (M1/70) that reacts with rat and human monocytes (Ault and Springer, 1981) was confirmed to be cytotoxic to human monocytes lysing 30% of each cell donor's monocytes but did not stain PTC endothelium.

renal endothelial cells. Thus, while all endothelial antigens may be expressed on monocytes, not all monocyte antigens appear to be expressed on all endothelial cells. Since these latter antigens may not be present in high concentrations in renal tissue, the use of monocytes alone for tissue typing and patient monitoring may yield results that do not fully correlate with renal graft survival.

Material and methods

Indirect immunofluorescence: Sections of frozen pretransplant biopsies from flushed, HLA-typed blood group O kidneys were fixed for 5 sec. in cold acetone before incubation with the primary serum. They were stained with fluoresceinated swine anti-human immunoglobulin or goat anti-mouse immunoglobulin (Nordic, Tilburg).

T-cell, B-cell and monocyte cytotoxicity: The standard NIH-lymphocytotoxicity assay was used for the detection of HLA–A, –B and –C antibodies and a cytotoxicity assay on nylon wool column enriched B-cells (Lowry et al. 1979) for the detection of DR and LB antibodies.

Monocytotoxicity was tested on monocytes enriched by adherence to plastic (Thompson et al. 1980). In brief, washed mononuclear cells from an isopaque-Ficoll density gradient (1.077), suspended in RPMI 1640 containing 20% human serum, were incubated in plastic Petri-dishes for 30 min at 37°C. After collecting the non-adherent cells, adherent cells were removed with lidocaine. The concentration of monocytes was in excess of 82% in the adherent fraction and less than 0.5% in the non-adherent aliquot. Carboxy-fluorescein labeled monocytes were incubated with serum for 60 min at 20°C. Following incubation with complement for 120 min at 20°C; 0.03% ethidium bromide in 2.5% EDTA NA_2 was added to stop the reaction and to label the nuclei of the dead cells.

Sera: Sera from 34 patients, which on preliminary screening contained antibodies to either endothelial cells or monocytes, were investigated for E–M reactivity. The 34 patients included: 25 Eurotransplant patients undergoing acute renal graft rejection, 2 Leiden patients undergoing acute bone marrow graft rejection, 5 Leiden volunteers who rejected skin allografts and 2 patients who had severe reactions to whole blood transfusions. In addition, the monoclonal antibody M1/70 was tested. M1/70 is a well-characterized rat monoclonal antibody which reacts with 190,000 and 105,000 dalton monomorphic determinants on mouse monocytes and cross-reacts with human monocytes (Ault and Springer, 1981).

Kidney Perfusions: Three human kidneys were studied which were unused by Eurotransplant because of surgical defects. After donor nephrectomy, the kidneys were immediately flushed with 500 ml of cold Euro-Collin's solution before an open recirculating perfusion system was established. The kidneys were flushed again with 50 ml of PBS and small wedge biopsies were "snap" frozen in isopentane on dry ice. Then the kidneys were perfused on ice for 2 h at a flow of 30–35 ml min with 50 ml serum from patient FM, or with 100 µl monoclonal antibody (M1/70) diluted to 50 ml in Euro-Collin's solution plus 2% bovine serum albumin. FM serum (500 ml) was obtained from a single plasmapheresis (24/10/79) of a patient who had high titers of circulating antibodies to E–M antigens.

Results

Sera from seven renal transplant recipients which had previously been demonstrated to

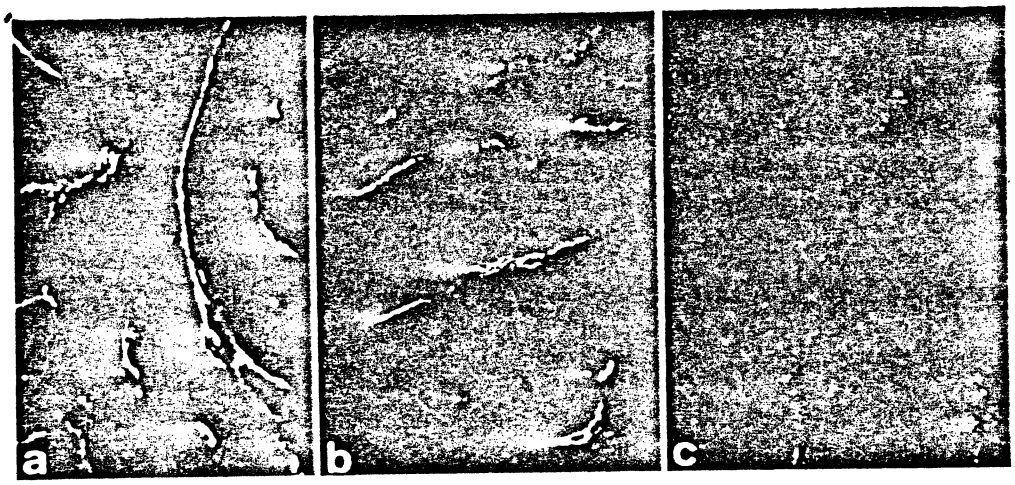

Figure 1. Immunofluorescent stains of kidney biopsy with 1:16 dilution of E–M positive serum following absorptions with (a) erythrocytes, (b) splenic lymphocytes, or (c) monocytes from the specific kidney donor.

To test whether these discordant reactions were attributable to differences in sensitivity between the two assays, two E–M positive sera were tested in doubling dilutions in the two assays, after HLA-reactive antibodies were absorbed with splenic lymphocytes. In both assays the unabsorbed sera were positive at dilutions of 1:32 – 1:128 and the absorbed sera were positive at dilutions of 1:16 – 1:32 (Table 2). Absorption with monocytes obtained from the same spleen completely removed the E–M reactivity in both assays (Figure 1). The sensitivity of the two assays also had been found to be similar for our other unabsorbed or partially absorbed E–M positive sera.

Since we have previously demonstrated that the endothelial reactivity of E–M antibodies can be removed by absorption with monocytes (Paul et al. 1979), we attempted to remove the monocyte reactivity of E–M antibodies by absorption to renal endothelial cells. This was accomplished by perfusing aliquots of E–M positive sera through the vessels of viable, healthy human kidneys, and then testing the sera for E–M activity following perfusion. Perfusion of transplant patient serum FM through a kidney that did not contain the relevant E–M antigen did not remove E–M reactivity. This demonstrated that E–M antibody activity was not lost through nonspecific trapping or dilution. Perfusion of a second aliquot of FM serum through a kidney with the appropriate E–M antigen removed its reactivity to the entire panel of 10 renal biopsies and 6 monocytes. Thus renal endothelium, like umbilical endothelium (Moraes & Stastny, 1977), can absorb antibodies to E–M antigens. However, when the monocyte-reactive M1/70 monoclonal antibody was perfused through a kidney, it retained monocyte reactivity, confirming its nonreactivity with renal endothelial cells.

Discussion

Our experience indicates that not all monocyte reactive antibodies are E–M antibodies. Ugolini et al. (1980) also have reported three

monoclonal antibodies that react with monocytes but not endothelial, B- or T-cells, and Thompson et al. (1980) have described a series of allo-antisera which react with monocytes and/or granulocytes but not endothelial cells. In addition, in a collaborative study with Gluckman et al. (1982) we found sera from 12 bone marrow transplant recipients which reacted with monocytes but failed to stain the PTC endothelium of 6 kidney biopsies. In the present study, only one of the two sera from bone marrow recipients with anti-monocyte reactivity also reacted with renal endothelium. Antibodies with monocyte reactivity in the absence of endothelial reactivity might be expected to be preferentially stimulated by bone marrow transplants since such transplants contain monocytic cell lines but few endothelial cells. Likewise, some renal transplant patients may be sensitized to monocyte antigens through blood transfusions or pregnancy rather than by the renal endothelium. However, even these methods of sensitization may stimulate E-M antibody formation sometimes, since typing sera from multiparous females and some unselected pregnancy sera contain E-M antibody reactivity (Baldwin et al. 1981).

Genetic studies indicate that there may be at least two monocyte-specific antigen systems; one which is linked to HLA and is expressed in association with β-2 microglobulin, and a second which is not linked to HLA or expressed in association with β-2 microglobulin (Stastny, 1978). We have found in preliminary family studies that the monocyte-reactive sera which also react with renal endothelial cells detect an HLA-linked antigen system, whereas those which do not react with renal endothelium detect a non-HLA-linked monocyte antigen system (Paul et al. [in press]). Although E-M antigens have a distribution on renal vessels which is similar to that of DR and LB antigens (Baldwin et al. 1981), E-M antigens are not expressed on resting B-lymphocytes as determined by cytotoxicity or absorption (Paul et al. 1979, Moraes and Stastny, 1977, Thompson et al. 1980). It is possible that E-M antigens are analogous to the newly described class IV major histocompatibility antigens of mice which in that animal are present in high concentrations in the cytoplasm of monocytes but are not expressed on the cell surface (Monaco and McDevitt, 1982).

The use of monocytes to screen for E-M antibodies also can be complicated by reactions of immune complexes with Fc receptors on monocytes. In our experience, sera containing immune complexes, especially those containing IgM or IgA immune complexes, can stain renal arterial or glomerular endothelium, but unlike E-M antibodies do not stain PTC endothelium strongly. These sera also can be cytotoxic to monocytes as can heat aggregated IgM and IgG (Baldwin and Claas, unpublished observations). Circulating immune complexes, however, do not correlate with graft rejection, but may be related to infection (Baldwin et al. 1982).

Finally antibodies which react with monocyte specific antigens but not endothelial cells could explain why antibody reactivity to monocytes does not always correlate as well with kidney graft rejection as does antibody reactivity to endothelial cells.

Acknowledgments

We thank Judith Burgers for excellent technical assistance and Mary Mentink for typing the manuscript. This work was supported by FUNGO grant 13-29-47.

W. M. Baldwin is a Juvenile Diabetes Foundation Research Fellow.

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Address:
Dr. W. M. Baldwin III
Nephrology Department
Academisch Ziekenhuis Leiden
Rijnsburgerweg 10
2333 AA Leiden
The Netherlands