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Reactivity of renal transplant sera against a 17 kD mononuclear cell antigen

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In recent years, studies have shown that non-HLA antigens can be involved in renal graft rejection [1]. The so called minor antigens have been found to be expressed on a variety of cells, including endothelial cells [2]. With the aim of understanding better the role of minor antigens in graft rejection, we undertook a Western blot screening of sera from patients waiting for or having received grafts from living-related or cadaveric donors. In this study we described the reactivity of these sera against a 17 kD antigen with expression in many different cell types.

Key words: Mononuclear cell antigen – Renal transplant sera

Methods

Forty-seven sera were studied, comprising sera from 20 patients on a waiting list for their first kidney transplant. 18 kidney transplanted patients, 6 heart transplant recipients and 3 patients who had received platelet transfusions. Of the total, 29 had high and 18 had low PRA (panel reactive antibodies).

Western blots were made according to Towbin et al. [3]. Lysates equivalent to 1×10^6 cells were loaded onto each gel slot, and separated in 5 to 15% gradients SDS-PAGE gels run under reducing conditions. The electrophoresed proteins were transferred to nitrocellulose filters overnight and incubated with the sera diuluted 1/40. The reactions were developed with anti-human IgG linked to peroxidase or alkaline phosphatase. Lysates were prepared from the following: T and B nylon wool-purified lymphocytes, Petri dish-adhered monocytes, blood platelets and erythrocytes, cultured fibroblasts from cell line (MRC-5), cultured endothelial cells from umbilical cord vein and liver, heart and kidney biopsies.

Specific antibodies to the 17 kD antigen were obtained by elution of the nitrocellulose filter after incubation with serum AT. The antibody elution protocol was performed according to Tovey and Baldo [4] and consisted of incubating a 0.5 mm wide strip of the filter containing the antigen and the antibody with 0.1 M Glycine, pH 2.8, for 2 min followed by brief centrifugation and neutralization with 1 M Tris buffer, pH 7.6. The eluted antibody was incubated with the CEM T cell line, followed by incubation with Protein A-Gold conjugate. The cells were processed for electron microscopy without any further staining.

In another set of experiments, mononuclear cell lysates on Western blot strips were submitted to sodium metaperiodate treatment at two molar concentrations: 10 and 100 mM. The blots were incubated in the dark, with one or other of the metaperiodate solutions for 10 min, followed by incubation with serum and color development. The 10 mM solution was used to oxidize and remove sialic acid residues from the proteins and the 100 mM eliminated galactose and other simple sugars.

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Results

1. Reactivity pattern

Sera from forty-seven patients waiting for or having received kidneys from living-related or cadaveric donors were submitted to Western blotting against mononuclear lysates from normal individuals. The sera were classified as high (greater than 50% Panel Reactive Antibodies PRA) or low (under 20% PRA) according to panel reactivity. The number of transfusions received by the individuals in each group varied greatly. In other words, high PRA sera from moderately-transfused patients as well as sera with low PRA from individuals with multiple transfusions were included for analysis. Positive reactions to a protein with an apparent molecular weight of 17 kD were frequent and usually of high intensity. The results are shown in Table 1 and Fig. 1. An increased frequency in

Table 1. Recognition of the 17 kD protein by 47 patients' sera. High (29) and low (18) PRA sera were tested. In both groups part of the sera recognized the specific-bound band by Western blot, although high PRA sera were more frequently reactive

	Panel reactivity	
	High (n)%	Low (n)%
17 kD +	(13) 68.4	(6) 31.6
17 kD –	(16) 57.1	(12) 42.9

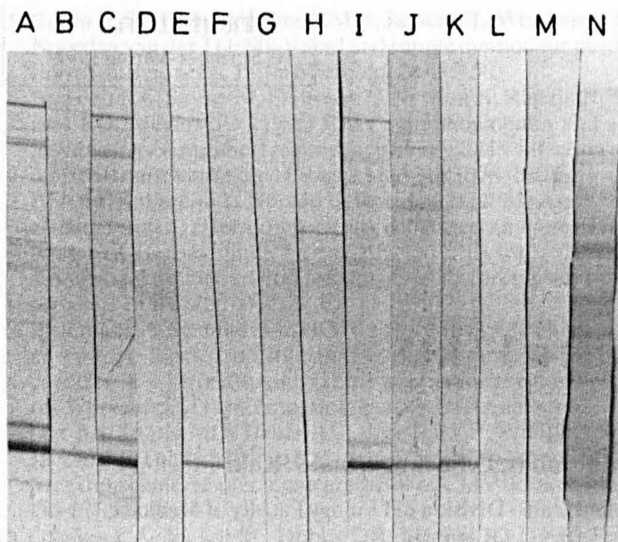

Fig. 1. Western blot using peripheral blood lymphocyte lysates as blotted antigen. From A to K: sera from transplanted kidney patients. Seven of the 11 sera were positive for the 17 kD antigen *(arrow)*. Lanes L and M are sera from normal controls. Lane N shows total PBL lysate

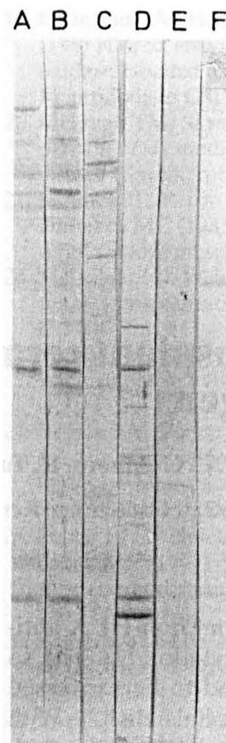

Fig. 3. Testing for heart recipient reactivity and for autoimmunity. In lanes A and B, sera of kidney recipients were tested against normal donors. In lane C the serum was from a heart transplant recipient, negative to the 17 kD antigen. Lanes D, E and F show an auto-Western experiment. In lane D, serum 1 reacts positively against cells from patient 2. In lane E, serum and cells are from patient 2 and reaction is negative and, in lane F, serum from patient 2 tests positive against third-party PBL

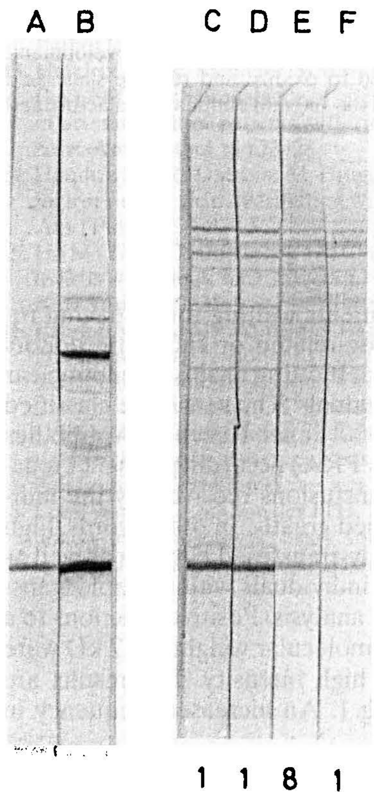

Fig. 2. Western blot with mono and multi-reactivity (A and B) and platelet absorption experiments (C to F). Lane A is a kidney recipient (AT) tested against normal PBL, showing monoreactivity compared to B where a multireactivity was observed. Lanes C and D showed reactivity before and after platelet absorption. Lanes E and F showed elimination of cytotoxicity after platelet absorption (8 to 1) with no change in reactivity to the 17 kD antigen

the high PRA group, though not statistically significant, was observed. In addition, comparison of graft survival rates in transplanted patients showed a slight increase in positivity to the 17 kD antigen of 84% (10/12) in the group of patients who lost their transplanted kidney, compared to 66% (4/6) in the group of patients with good outcome.

Six serum samples from heart-transplanted patients, followed for over a year, were negative for the 17 kD antigen. These patients had not received any transfusion prior to grafting, according to the heart transplant protocol established at our Institution. However, the number of transfusions received did not correlate with percentage of positivity of sera to the 17 kD antigen in the high and low groups. Additionally, three patients who received platelet-rich transfusions exhibited the antibody against the 17 kD antigen on mononuclear cells. Finally, there was no correlation between the presence of the anti-17 kD antibody and AB0 blood groups of the patients.

Platelet-absorbed sera did not change their reactivity to the 17 kD antigen (Fig. 2). Besides, serum samples collected on different dates from the same patient (three different patients), with high and low reactivity always exhibited the same pattern and level of reaction irrespective of the PRA level.

2. *Nature of the anti-17 kD antibody*

Reaction on Western blots was consistently developed with anti-IgG conjugates. Thus, in accordance with the nature of the antibody, cytotoxicity assays against mononuclear cells were performed simultaneously using unabsorbed and platelet-absorbed sera. Reactivity to the 17 kD antigen was never removed, even when absorption led to loss of the cytotoxicity, thus indicating that this antigen was not involved in cytotoxic processes (Fig. 2). Reactivity to the 17 kD antigen was unchanged after treatment with two molar concentrations of sodium metaperiodate (data not shown), indicating that the antibody was directed against the protein structure and not the glycosilation residues.

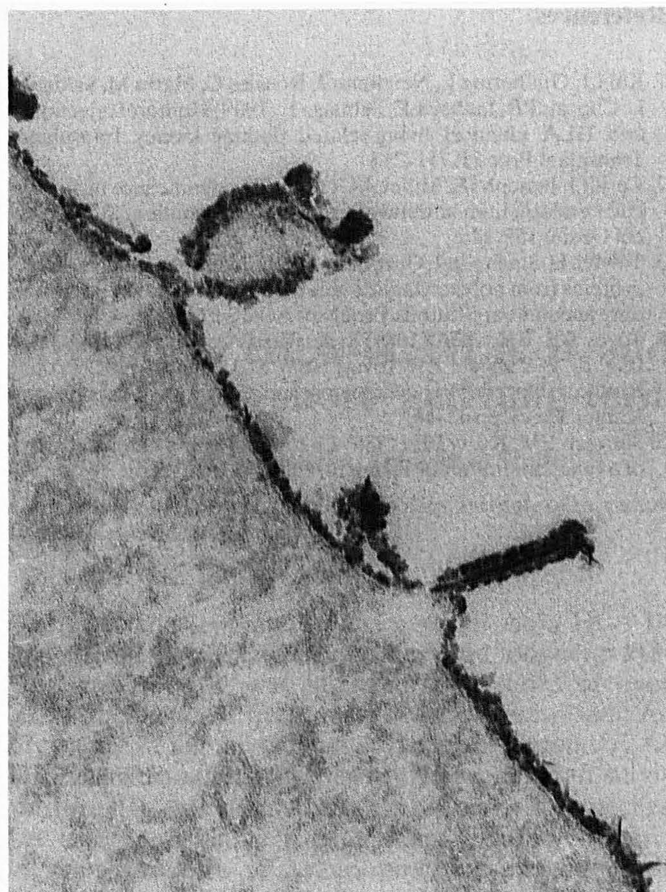

Fig. 4. Electron microscopy of CEM T cell line. Specific antibodies to the 17 kD antigen were eluted after reacting with the band on nitrocellulose filters. After incubation with fixed CEM T cells, reactivity was developed with colloidal gold conjugated second antibody

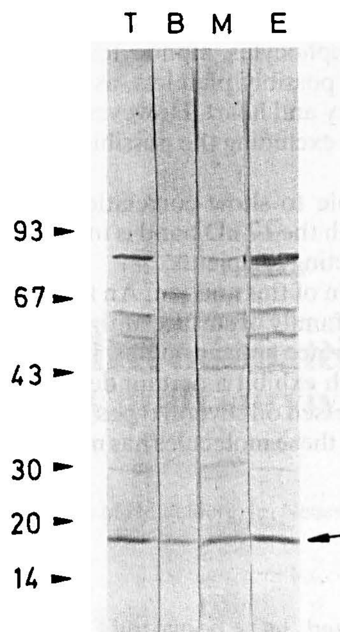

Fig. 5. Western blot with different cell types from the same donor. Serum from a renal transplant recipient was tested against T and B lymphocytes, monocytes and endothelial cells from the same umbilical cord. The *arrow* points out the 17 kD antigen

3. *Expression of the 17 kD antigen*

Sera from four positive patients were submitted to an auto-Western blot and were shown to be negative (see Fig. 3). In order to confirm the presence of this antigen on the cell membrane, specific antibodies were eluted from the nitocellulose filter and were incubated with the CEM T cell line. Incubation with protein A–gold conjugate followed and the resulting reaction was observed with an electron microscope. The photomicrographs showed a linear staining on the cell membrane, even in finger-like protusions of the cytoplasm. No intracellular structures were stained (see Fig. 4).

Finally, sera with strong positive reactivity were analyzed by Western blotting against several types of cells. In one experiment serum was incubated with lysates from T and B lymphocytes, monocytes and umbilical vein endothelial cells from the same donor. Reactivity was equivalent in the four types of cells as shown in Fig. 5. Table 2 shows the results obtained with cells from several different tissues.

Table 2. Cell distribution of the 17 kD antigen. Erythrocytes and liver cells were the only negative cells tested so far

Type of cell	Presence of 17 kD antigen
T lymphocytes	+
T lymphocytes, activated	+ [a]
B lymphocytes	+
Monocytes	+
Endothelial cells	+
Kidney	+
Heart	+
Liver	–
Platelets	+/–
Fibroblasts	+
Erythrocytes	–

[a] T lymphocytes, fresh and cultured for 3 days in the presence of phytohemagglutinin, were analyzed simultaneously showing no change in intensity of band staining

Discussion

In order to identify new cell antigens recognized by transplant recipient sera, we studied the reactivity of selected sera against cell lysates by Western blotting. In the course of this screening, an antigen of 17 kD was identified and recognized by 19 of 47 sera tested (40%). The reactivity was elicited by allostimulation since non-transfused non-transplanted donors do not react with this band. Blood transfusion seems to be the most common way of immunization, high PRA individuals presenting with the antibody more frequently. It is not an autoantibody since positive sera repeatedly do not recognize the antigen on autologous cells. Nevertheless, a putative polymorphism of the system was not assessed so far and further analyses are needed. Electron microscopic immuno-staining with protein A showed uniform distribution of the antigen on the surface of the CEM T cell line. Paradoxically, antibodies to the 17 kD protein did not cause complement-dependent cell lysis.

The distribution of this molecule was widespread, being found on T and B lymphocytes, monocytes, endothelial cells, fibroblasts and possibly platelets, as well as diverse tissues such as kidney and heart. However, it was not present on erythrocytes, excluding the possibility of a blood type antigen.

Although we were unable to show correlation with graft rejection, reactivity with the 17 kD band is most frequently present in graft rejecting recipients.

We do not know the origin of this antigen. An analogy may be drawn with the Ly-6 family of surface antigens [5]. This family comprehends surface antigens of low molecular weight (12–18 kD), which exhibit a certain degree of polymorphism and are expressed on several types of cells. As in our case, a function for these molecules has not been found.

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