Humoral Response of Miniature Swine Tolerant to Class I Mismatched Renal Allografts

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IT HAS BEEN previously shown in our miniature swine model that the use of high-dose Cyclosporine A (CyA), 10 mg/kg per day for 12 days, is sufficient to induce specific and permanent tolerance to class II matched but class I mismatched allografts; ie, SLA^{gg} (I^c, II^d) into SLA^{dd} (I^d, II^d).[1] In addition, a second kidney transplant from the same or from an SLA-matched donor when performed 6 months after the first transplantation was also permanently accepted without additional treatment.[2] Untreated animals rejected and produced IgM and IgG antibodies (Ab) against donor type cells that were cytotoxic and directed predominantly against donor class I antigens. In contrast, approximately 80% of tolerant pigs (10 of 13) developed noncytotoxic IgG Ab directed against non-SLA class I antigens, and no significant IgM response. The present report focuses on preliminary biochemical analysis of these humoral responses.

MATERIALS AND METHODS

Purification of Noncytotoxic IgG (NcIgG)

The IgG fractions from sera 10625 (Rejector) and from 10349 (Tolerant) were isolated using a DEAE Sephadex A-25 column as described.[3] Specific NcIgG were further enriched by specific binding to donor type PBMC followed by elution with 0.1 mol/L citrate buffer (pH = 3).

Radiolabeling and Immunoprecipitation

Biosynthetic labeling of proteins with ^{35}S methionine was carried out for 6 hours in methionine-free medium. Cells were lysed in buffer containing 20 mmol/L Tris HCl, pH = 8; 120 mmol/L NaCl; 0.2 mmol/L EGTA; 0.2% desoxycholate (DOC); protease inhibitors, and 0.5% NP40. Cell lysates were pre-cleared by using normal pig serum (NPS) and Protein A-Sepharose CL-4B. Immune complexes were resolved by SDS-PAGE in reducing conditions.

RESULTS AND DISCUSSION

Noncytotoxic (Nc) Ab, of IgG isotype, purified on donor type PBMC from the tolerant animal 10349, were used to immunoprecipitate the antigen(s). SDS-PAGE analysis presented in Figure 1 clearly showed that NcAb bind to a cytosolic molecule of approximately 180 kD and referred to as AgX (compare lane 2 to lanes 1, 3, 4, 5, and 6). In contrast, Ab isolated from the rejecting animal 10625 did not permit detection of this molecule (lane 4). An alloantiserum, directed against pig class I, precipitated a molecule of 44 kD corresponding to the SLA class I heavy chain (lane 6). Both the membrane and the cytoplasmic forms of AgX had a similar molecular weight, strongly suggesting that the AgX did not undergo major posttranslational modifications which could have modified either its conformation and/or antigenicity (data not shown). Taken together, these data suggested that the AgX might be a marker of tolerance status. However, an analysis of the distribution of AgX among the herd with respect to SLA haplotypes showed that AgX was detected in some animals of all the SLA

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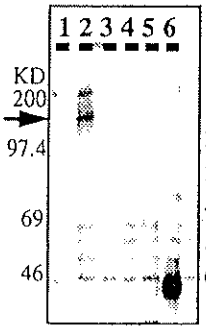

Lane #	Ab Source	Affinity Purified on	Combined to cell extracts from
1	no Ab		AgX^+ PBMC
2	Ab from tolerant (10349)	AgX^+ PBMC	id
3	id	AgX^- PBMC	id
4	Ab from rejector (10625)	AgX^+ PBMC	id
5	id	AgX^- PBMC	id
6	Pig serum anti-class I		id

Fig 1. SDS-PAGE analysis of Antigen/Ab complexes between ^{35}S-proteins and NcAb. Immunoprecipitations of AgX, isolated from radiolabeled PBMC, were resolved by SDS-PAGE under reducing conditions. Bands were revealed by autoradiography. The arrow marks the band corresponding to AgX. (id = same as above)

haplotypes, suggesting that synthesis of NcAb against AgX is not a property of tolerant animals but rather that NcAb can be elicited against the AgX molecule when donor/recipient combinations permit. Experiments are in progress to characterize and define further the structural features of AgX that result in the exclusive production of NcAb.

REFERENCES

1. Rosengard BR, Ojikutu CA, Guzzetta PC, et al: Transplantation 54:490, 1992

2. Rosengard BR, Fishbein JM, Gianello PR, et al: Transplantation 57:794, 1994

3. Corthier G, Boschetti E, Charley-Poulain J: J Immunol Methods 66:75, 1984