Xenogeneic T-Cell–Mediated Immune Reactivity in the Model of Pig-to-Humans: First Findings With Native Stimulator Cells

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COMPARED with the humoral response in xenotransplantation, which is dominated by preformed natural and graft-induced xenophile antibodies, and which represents the primary barrier to successful transplantation, much less is known about the cell-mediated xenogeneic immune response. Two suggestions have caused confusion rather than clarity: (1) the cell-mediated response is either low or even lacking, because costimulatory cell surface molecules on stimulator and responder cells are incompatible and because humoral costimulating factors are species specific,[1] and (2) the response is significantly lower than the allogeneic response because xenoantigens are exclusively presented via the indirect pathway by the host's antigen-presenting cells (APC).[2] However, there is a single report documenting direct xenoantigen presentation to human $CD4^+$ T cells, including porcine, rabbit, monkey, dog, and rat antigens, but excluding mouse antigens.[3] The aim of this study is the evaluation of the capability of porcine MHC class II^+ APC to directly trigger a T-cell–mediated xenogeneic immune response in the potential future recipient of porcine donor organs, the human patient.

MATERIALS AND METHODS

Preparation of Peripheral Blood Lymphocytes (PBL) That Serve as Responders and Stimulators in the Mixed Lymphocyte Culture (MLC)

Human PBL. Heparinized vein blood was obtained from two healthy, HLA-typed human volunteers, H1 (man, 35 years) and H2 (woman, 40 years). PBL were prepared by density gradient centrifugation according to a standard protocol and adjusted to a final concentration of 5×10^4 cells/mL RPMI supplemented with 10% heat-inactivated human AB serum. These cells served as responders (unseparated and separated: see below) in the xenogeneic and the allogeneic MLC, and also as stimulators in the allogeneic MLC. Stimulator cells were irradiated with 25 G.

Porcine PBL. Heparinized porcine blood was collected from pigs at a local slaughterhouse during the slaughtering process under semisterile conditions. PBL were prepared from whole blood according to the protocol for human cells (see above). The final cell concentration was 5×10^4 cells/mL RPMI supplemented with 10% heat-inactivated human AB serum. These cells served as stimulators (unseparated and separated: see below) in the MLC and were therefore irradiated with 25 G.

Xenogeneic MLC. The xenogeneic MLC consisted of two experiments, H1-anti-pig and H2-anti-pig. The MLC (coincubation of 5×10^4 responders and 5×10^4 irradiated stimulators) lasted for 6 days (37°C, 5% CO_2 in air, humidified). Cell proliferation was measured by ^{3}H-thymidine incorporation during the last 16 hours of the culture period.

Allogeneic MLC. The allogeneic MLC, H1-anti-H2 and H2-anti-H1, served as allogeneic control and proliferation reference. The combination was mismatched at one A locus, two B loci, and one DR locus. The number of responder and stimulator cells, culture time, and ^{3}H-thymidine uptake were otherwise identical to the xenogeneic experiment.

Cell Separation Procedure. To determine the pathway of antigen recognition or antigen presentation (direct vs indirect), responder and stimulator cells were separated from potential APC. Human T cells were prepared from PBL by separating B lymphocytes (incubation with anti-CD19 monoclonal antibody [MAb], Dianova, Hamburg, Germany) and monocytes (anti-CD14 MAb, Dianova) with the help of magnetic, secondary antibody-coupled dynabeads (Dynal, Oslo, Norway). APC-free porcine T cells were prepared from PBL by separating the monocytes/macrophages with the mouse anti-pig MAb, 74-22-15A, (ATCC, Rockville, Md) and coupling them to dynabeads (see above), leaving B cells unaffected. They did not act as APC because of the purification control experiment: human T-anti-porcine T gave negative proliferation results. The quality of the cell separation, whether human or porcine, was additionally controlled by the indirect immunofluorescence binding assay using flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany). Human responder T cells and porcine stimulator T cells were used in coculture experiments when APC contamination was 0.09% or less.

RESULTS

The results of this study investigating the cell-mediated xenogeneic immune response in the clinically relevant combination human anti-pig are documented in Fig 1. The results for H1-anti-pig are shown in the top part of the figure and the results for H2-anti-pig in the bottom part. The main findings are: (1) When unseparated human responder PBL are cocultured with unseparated, irradiated native porcine stimulator PBL in the xenogeneic MLC, the human T cells show a significant cell-mediated immune response against porcine antigens. This holds for both responders, H1 and H2 (experiment 2). In both cases the xenogeneic responses are similar in strength to the allogeneic MLC control responses (experiment 1). (2) When purified human T cells serve as responders to nonpurified porcine stimulator cells, thus analyzing the direct pathway of antigen presentation and eliminating the indirect pathway, different results are observed with the two human responders: (a) a significantly reduced response with responder H1 (experiment 3, top), indicating a

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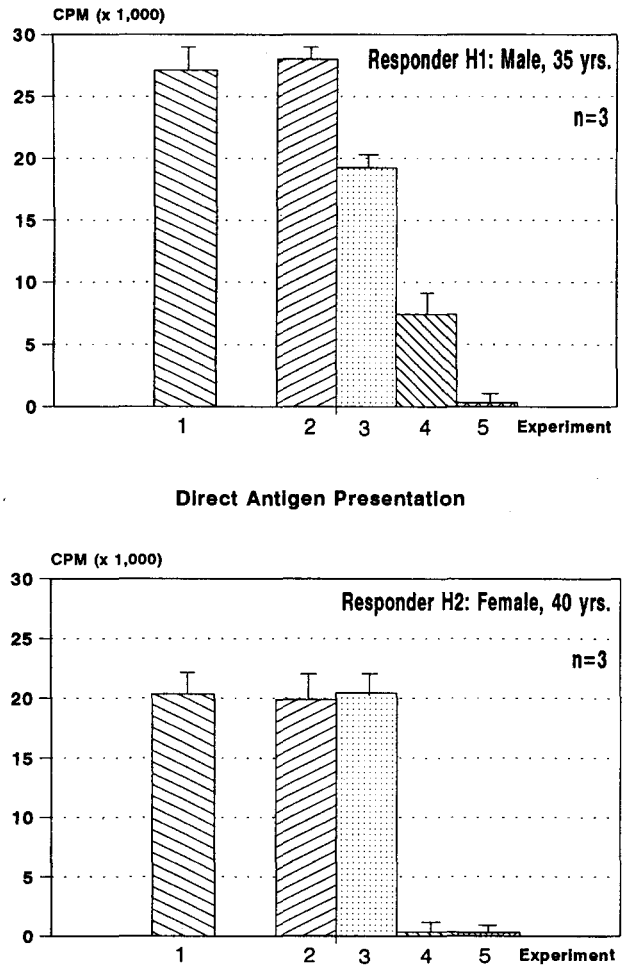

Fig 1. Xenogeneic T-cell-mediated immune reactivity in the model pig-to-human. Xenogeneic MLC with human PBL as responders and irradiated native porcine PBL as stimulator cells. The top part of the figure shows the proliferation results with human responder H1, the bottom part the results with human responder H2. Experiment 1: Allogeneic control response H1-anti-H2 (top) and H2-anti-H1 (bottom). Experiment 2: Xenogeneic response H1-anti-pig (top) and H2-anti-pig (bottom) using unseparated human and porcine PBL. Experiment 3: As in experiment 2, but with APC-free, purified human responder T cells (minus $CD14^+$ monocytes and $CD19^+$ B lymphocytes) to analyze direct antigen presentation. Experiment 4: As in experiment 2, but with APC-free, purified porcine stimulator T cells (minus 74-22-15A^+ monocytes) to analyze indirect antigen presentation. Experiment 5: A combination of experiments 3 and 4, to functionally confirm that the purification status of responder T and stimulator T cells is satisfactory.

partial lack of direct presentation and (b) the full response with responder H2 (experiment 3, bottom), indicating direct presentation only. (3) When purified porcine T cells, which were shown to be incapable of stimulating human responder T cells in a control assay (experiment 5), were cocultured with human PBL, thus excluding direct presentation, the results obtained with the two human responders differed again: (a) a moderate but significant response was obtained with responder H1 (experiment 4, top), indicating indirect antigen presentation, and (b) a completely negative response with responder H2 (experiment 4, bottom), indicating the lack of indirect antigen presentation by this responder's APC. These reaction patterns have been confirmed with other human responders (data not shown).

DISCUSSION

This report deals with work still in progress. The main finding, a significant difference in the responsiveness of human T cells against porcine antigens, has been confirmed with several individuals in each T-cell–mediated reaction pattern group. The reason why human T cells recognize porcine antigens either entirely directly or in a mixed fashion, that is, directly and indirectly, is not yet understood and will be further evaluated. If the two reaction patterns in Fig 1 could be confirmed with a larger number of human responders, this would definitely have consequences for the necessity of manipulating xenograft immunogenicity prior to transplantation.

It appears to be clear at this point that an allogeneic T-cell–mediated response is much easier to induce than the xenogeneic response human anti-pig. This may be concluded from cell separation experiments with insufficiently purified T cells. Whereas such a batch of human responder T cells was still capable of eliciting a significant immune response against sufficiently purified allogeneic T-stimulator cells (controlled by FACS), they were incapable of eliciting a response against sufficiently purified porcine T cells (data not shown). However, if a xenogeneic response was inducible, its magnitude was similar to the allogeneic response. While we were preparing this manuscript, our findings were confirmed by other authors.[4] The basic findings here and there are comparable: a strong xenogeneic response human anti-pig that more or less equals the human allogeneic response. As our understanding of the basic mechanisms of cellular immunity in this xenogeneic combination with its clinical potential grows, the time may not be too far away that porcine tissue will help to solve the problem of human donor organ shortage.

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