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# Destruction of Endothelial Cells by Humoral Factors Obtained From Recipients of Xenografts

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THE MODERATELY difficult model of hamster to rat xenotransplantation has been used by many investigators to study the effects of different immunosuppressive regimens.[1-3] Our previous studies have shown that without treatment the graft survival times in this xenotransplant model were 3 days, 6 days, and 7 days for heart, kidney, and liver xenografts, respectively.[1-4] We and others have also shown that in this model heart and kidney xenografts were rejected mainly by the humoral factors whereas the liver was rejected by a mixture of cellular and humoral factors.[1-4] There are two known mechanisms whereby humoral factors can facilitate the destruction of xenografts, namely C'-dependent Ab-mediated cytotoxicity (CDC) and Ab-dependent cell-mediated cytotoxicity (ADCC).[1,5] The purpose of this study was to analyze the induction of humoral factors after heart, kidney, and liver xenotransplantations using hamster aortic endothelial cells (AEC) as targets for CDC and ADCC assays rather than the conventional lymphocyte targets.

## MATERIALS AND METHODS

Methods for organ transplantation,[2-4] isolation, and culture of AEC[6] have been described previously. The medium used for AEC cultures was DMEM/F12 containing 5% to 10% hamster serum. $^{51}Cr$ release assay[7] was used to measure the cytotoxic humoral factors against AEC (2 hours for CDC, 4 hours for ADCC). The ADCC was expressed as the difference between percent specific release in the presence of serum and effector cells and percent specific release in serum alone.

## RESULTS AND DISCUSSION

Histologic study of xenograft rejection indicates that the vascular endothelial cell (VEC) is a major target for cytotoxic humoral factors in xenograft rejection.[8] In vitro studies have also shown that the VEC is central to xenogeneic immune reactivity.[5] Therefore, the conventional method using lymphocytes as targets for measuring cytotoxic serum factors may not be as relevant as using VEC as targets. Table 1 shows the kinetics of CDC and ADCC Ab induction after xenografting, using hamster AEC as target cells. The pretransplant (tx) rat sera contained a small amount of natural Ab against hamster AEC in the CDC assay, the titer disappearing after 1:40 dilution. The same serum did not facilitate the target cell killing in the ADCC assay, since killing by effector cells alone was 10.1 ± 2.1% (Mean ± SEM of five experiments). For heart and kidney

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0041-1345/94/$3.00/+0

**Table 1. Destruction of AEC by Humoral Factors Obtained From Xenograft Recipients**

| Sera | Assay | Percent Specific Release at Various Serum Dilutions* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 |
| Pre-Tx | CDC | | 14.3 ± 7.8 | 2.9 ± 2.1 | 0 | 0 | 0 | 0 |
| | ADCC | 6.1 ± 2.7 | 5.9 ± 1.5 | 6.8 ± 1.1 | 7.3 ± 0.9 | 7.8 ± 1.1 | 8 ± 0.7 | |
| HTD2 | CDC | | 56.8 ± 12.6 | 48.2 ± 3 | 29.2 ± 9.4 | 17.1 ± 11.9 | 11.6 ± 11.3 | 5.8 ± 4.6 |
| | ADCC | 7.6 ± 1.1 | 6 ± 0.2 | 3.1 ± 1.1 | 2.3 ± 0.6 | 2 ± 0.6 | 1.3 ± 0.5 | |
| HTD3 | CDC | | 68.3 ± 3.7 | 64.7 ± 7.7 | 60.2 ± 6.6 | 50 ± 8.5 | 48.2 ± 9.7 | 38.8 ± 12.8 |
| | ADCC | 53.7 ± 27 | 36.5 ± 12.6 | 20.8 ± 0.8 | 12.1 ± 3.7 | 8.4 ± 3.6 | 4.3 ± 0.1 | |
| KTD3 | CDC | | 51.8 ± 8.6 | 40.3 ± 7 | 21.8 ± 0.9 | 14.7 ± 8.3 | 3.3 ± 4 | 0.7 ± 0.8 |
| | ADCC | 15.2 ± 1.6 | 10.8 ± 0.1 | 10.4 ± 2.1 | 9.9 ± 4.1 | 10.1 ± 4.7 | 10.3 ± 5.6 | |
| KTD5 | CDC | | 39.7 ± 15.1 | 55.2 ± 12.7 | 61.1 ± 11.9 | 63 ± 13 | 60 ± 12.3 | 53.4 ± 17.1 |
| | ADCC | 42.5 ± 9.8 | 32.8 ± 11.7 | 22.9 ± 7.5 | 20 ± 7.2 | 17.6 ± 6.2 | 15 ± 4.9 | |
| LTD3 | CDC | | 30.2 ± 12 | 25.5 ± 12.7 | 21.1 ± 13 | 17.3 ± 5.7 | 14.7 ± 12.9 | 11.3 ± 11.3 |
| | ADCC | 4.9 ± 2.3 | 7.4 ± 3 | 8.5 ± 3.9 | 8.7 ± 4 | 8.8 ± 4.6 | 7.8 ± 2 | |
| LTD5 | CDC | | 74.6 ± 12.8 | 75.9 ± 10.5 | 76.6 ± 10.3 | 73.9 ± 9.8 | 68.1 ± 7.6 | 65.1 ± 7.3 |
| | ADCC | 8.5 ± 1.7 | 13.1 ± 6.9 | 13.9 ± 7.8 | 10.4 ± 5 | 12.6 ± 7.3 | 11.2 ± 6.3 | |
| LTD7 | CDC | | 47.1 ± 5 | 49.3 ± 3.9 | 47.4 ± 6.9 | 48.3 ± 7.9 | 45.9 ± 9.4 | 40.3 ± 6.6 |
| | ADCC | 20.6 ± 9.3 | 24.3 ± 13 | 26 ± 13.8 | 26.7 ± 15.3 | 25.1 ± 14.7 | 17 ± 7.9 | |

*Mean ± SEM of three experiments.

xenografts, CDC Abs in the sera gradually increased and peaked before the grafts were rejected. However, in liver xenograft the titer of CDC Ab increased until 5 to 6 days post-tx; then the titer dropped before the graft was rejected, suggesting that most of the anti-VEC Abs probably had already adsorbed to VEC in vivo. It is of interest to note that the same sera did not show the reduction in Ab titer on day 7 post-tx when lymphocytes were used as targets for the CDC assay (data not shown). This finding confirms the notion that Ags presented on AEC and Ags on lymphocytes that are recognized by the same sera are not identical.[5,9] The Ab mediating CDC can be dissociated by addition of dithiothreitol, indicating the Ab is of IgM isotype (data not shown).[10]

Although all three xenografts induced a high titer of CDC Ab against AEC, the same sera were found to be ineffective in the ADCC assay. Results in Table 1 show that only day-3 sera after heart tx and day-5 sera after kidney tx mediate some measurable amount of cytotoxicity. Day-5 sera from liver tx mediated the highest level of CDC; however, the same sera had no effect in the ADCC assay, suggesting that these two mechanisms of killing are mediated by different Ab. In conclusion, we have demonstrated that cytotoxic humoral factors against AEC were induced after xenotransplantation in this model.

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