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Cytokine Messenger RNA Profiles in Hamster-to-Rat Liver Xenografts

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XENOGENEIC transplant rejection is thought to be mediated primarily by a humoral mechanism. Discordant xenografts are rejected hyperacutely by preformed natural antibodies recognizing genetically disparate donor cells. Concordant xenografts are also destroyed by antibodies; however, this rejection process takes place over a longer span of time, during which T-helper cells play a crucial role in mounting the antibody response.[1] The concordant xenograft antibody response contains two components: one results in an immunoglobulin M (IgM) response and is primarily T cell independent, whereas the other results in an IgG response and is T cell dependent.[2] This helper cell–evoked antibody response is presumably due to helper cell production of B-cell growth factors such as interleukin-4 (IL-4), IL-6, and IL-10. To investigate the immune mechanism underlying the xenogeneic response, we used the hamster-to-rat liver xenograft model. After transplantation, serum antibody titers of both IgM and IgG were markedly elevated as early as day 3 and peaked around day 6. These grafts invariably reject by day 7 posttransplant with histologic findings suggestive of antibody-mediated and cellular-mediated injury. The presence of high levels of IgM and IgG in our hamster-to-rat animals suggested involvement of T cells in the xenograft immunity. To determine the pattern of T-helper activity, we examined cytokine messenger RNA (mRNA) profiles in liver xenografts.

METHODS

Liver grafts from Syrian Golden hamsters were transplanted into male Lewis rats using the cuff technique described by Kamada.[3] No immunosuppressants were administered, and animals were killed on day 5 postgrafting. Total RNA was extracted from liver grafts using RNAzol (Cinna/Biotecx) and quantitated spectrophotometrically. Five micrograms of RNA was reverse transcribed into cDNA and 1-μg aliquots subjected to polymerase chain reaction (PCR) amplification for 25 cycles using ^{32}P-labeled primers specific for rat cytokines and glyceraldehyde-3-phosphate dehydrogenase (G3PD), a housekeeping gene. Quantitation of amplification product was performed on a radioanalytic scanner after polyacrylamide gel electrophoresis. Specificity of amplification was confirmed by sizing and restriction analysis.

RESULTS AND DISCUSSION

Examination of cytokine mRNA levels in hamster-to-rat liver xenografts using rat-specific radiolabeled reverse-transcription PCR (RT-PCR) demonstrates the presence of detectable recipient rat T-helper cell cytokine mRNAs (Table 1) at day 5 posttransplant. We were more easily able to detect IL-4 and IL-10 mRNA than either IL-2 or interferon-γ (IFN-γ) mRNAs. Helper T cells are divided into two major subsets based on their pattern of cytokine production.[4] The T-helper 1 (TH1) subset of cells is characterized by the production of IL-2 and IFN-γ and are responsible for the induction of the delayed-type hypersensitivity. In contrast, the T-helper 2 (TH2) subset of cells is characterized by the production of IL-4, IL-5, IL-6, and IL-10. Because IL-4, IL-6, and IL-10 are potent B-cell growth factors, the TH2 subset is thought to mediate humoral immunity. The vigorous humoral response observed after xenotransplantation and our ability to easily detect the TH2 cytokines IL-4 and IL-10 suggest that TH2 cells may play an important role in mediating xenograft rejection. Whether TH2 activity predominates over TH1 activity in the xenograft remains to be determined; direct comparison between cytokines measured by PCR cannot be made unless the amplification efficiency for each cytokine is determined and taken into account. We have previously shown in vitro with xenogeneic mixed lymphocyte response (MLR) (mouse–anti-rat) that there is a high expression of TH2 cytokine mRNAs.[5] In contrast, mouse–anti-mouse allogeneic MLR showed a relatively higher expression of TH1 cytokine mRNAs using the same assays. These previous findings indicated that TH2 cells may

Table 1. Cytokine mRNA Levels Detected by Radiolabeled Reverse-Transcription PCR in Hamster-to-Rat Liver Xenografts

Cytokine	Grafts A	Grafts B	Grafts C	Mean Counts
IFN-γ	259	80	280	206
IL-2	87	272	89	149
IL-4	3923	381	1,661	1,988
IL-10	13,976	3,351	11,132	8,765
G3PD	108,840	60,480	87,720	85,680

LVG hamster livers were transplanted into Lewis rat recipients and the xenografts harvested on day 5 posttransplant. One-microgram aliquots of RNA were subjected to reverse-transcription PCR amplification for 25 cycles using ^{32}P-labeled primers targeted to rat cytokines and G3PD. Quantitation of amplification product was performed on a radioanalytic scanner after polyacrylamide gel electrophoresis. Data from a representative experiment are expressed above in total counts of radioactivity over background.

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differentially respond more during the xenogeneic than allogeneic response. Although it is possible that our MLR findings were due to the necessarily artificial conditions in vitro, our current findings in vivo with hamster-to-rat xenografts are also consistent with increased TH2 activity relative to TH1 activity in xenogeneic responses. The reason for increased TH2 reactivity after xenografting is unknown, but ligand binding by responding T cells may play a role. Bottomly has shown that high antigen ligand density binding of T-cell receptors leads to a predominantly TH1 reaction, whereas low ligand density TCR binding leads to a predominantly TH2 reaction.[6] After xenografting, poor interaction between accessory molecules due to species differences may interfere with the normally high-affinity T-cell receptor binding and thus effectively decreases perceived antigen ligand density.

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