Transpl Int (1998) 11 [Suppl 1]: S357–S360

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Soluble donor MHC class I antigen inhibits immunologic priming in vitro and in vivo

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Abstract Previous studies suggest liver transplants can protect other transplanted organs. This effect may be mediated by hepatocytes secreting large amounts of soluble MHC class I antigen. Here our aim was to determine whether immunologic priming by membrane-bound alloantigen could be inhibited by allospecific antigen produced in a secreted form. Cultured Lewis (RT1.A^l) hepatocytes were transfected with plasmids encoding either a membrane-bound or secreted form of the alloantigen, RT1.A^a. Cytotoxic T-lymphocyte (CTL) precursor assays were performed on Lewis splenocytes cultured with transfected hepatocytes, or hepatocytes were injected into the portal vein of prospective Lewis recipients of an ACI (RT1.A^a) liver allograft. Results showed CTL priming by membrane-bound RT1.A^a was inhibited in vitro by soluble RT1.A^a. Similarly, acceleration of ACI allograft rejection induced by membrane-bound antigen was abrogated following co-injection of hepatocytes secreting donor alloantigen. In conclusion, production of soluble donor alloantigen by liver transplants may provide protection against the alloimmune response.

Key words MHC · Hepatocyte · CTL · Gene transfer · Liver

Introduction

There is a substantial amount of data that suggests liver transplantation can have potent immunosuppressive effects [1, 2, 10]. Different hypotheses have been put forth to explain this phenomenon. One potential explanation relates to the ability of the liver to produce large quantities of soluble MHC class I antigen under normal physiologic conditions, and following liver transplantation [1, 2]. This theory is supported by in vitro studies that suggest soluble alloantigen can downregulate different aspects of the immune response, including antibody responses [8], and helper and cytotoxic T cell responses [3, 12]. In vivo, Sumimoto and Kamada [11] have reported that purified soluble donor MHC class I antigen complexed with alloantibody can prolong heart allograft survival. In addition, we have recently reported that genetically modified autologous hepatocytes made to express soluble donor MHC class I antigen can prolong liver allograft survival [6].

Previously we have shown that autologous hepatocytes transfected with plasmid DNA encoding membrane-bound alloantigen have a sensitizing immunologic effect when injected in vivo, as evidenced by CTL priming and acceleration of allospecific heart and liver allograft rejection [6, 7]. In the present study we used the same hepatocyte gene-transfer system to determine the ability of soluble allo-MHC antigen to inhibit immunologic priming induced by this membrane-bound alloantigen. Autologous hepatocytes were transfected with plasmid DNA encoding membrane-bound alloantigen to prime cytotoxic T-lymphocytes (CTL) in vitro, and to accelerate liver allograft rejection when injected via the portal vein. To determine if CTL priming could be inhibited by secreted alloantigen, splenocytes were cultured with autologous hepatocytes co-transfected

with plasmid DNA encoding membrane-bound and secreted allo-MHC antigen. Similar studies were conducted in vivo to determine if immunologic priming could be reduced by injecting a mixture of recipient-strain hepatocytes expressing either membrane-bound or secreted donor MHC antigen via the portal vein, prior to liver transplantation.

Materials and methods

Hepatocyte culture and transfection

Viable hepatocytes were obtained from Lewis (RT1.A^{l}) rat livers by a two-step perfusion system using a collagenase H (Boehringer Mannheim, Indianapolis, Ind.) solution. Washed hepatocytes were purified using a Percoll gradient as previously described [7]. Purified hepatocytes were cultured for 2 days on collagen-coated (Collagen Corporation, Palo Alto, Calif.) petri dishes. Cells were then transfected with plasmid DNA by lipofection (Lipofectin; Life Technologies, Gaithersburg, Md.), as previously described [7]. Two RT1.A^{a}-encoding plasmid DNA constructs were used: (1) pcRT.45, encoding the membrane-bound form of RT1.A^{a}; and (2) pcRQ.B3, encoding a secreted, soluble form of RT1.A^{a}. The DNA constructs and specific expression of membrane-bound or secreted RT1.A^{a} products by hepatocytes transfected with each plasmid have been described in detail elsewhere [5, 6].

In vitro experiments

Lewis splenocytes were obtained and co-cultured for 2 days with hepatocytes transfected with: (1) control plasmid (pCMVLux, encoding firefly luciferase); (2) pcRT.45; or (3) both pcRT.45 and pcRQ.B3. Conditioned splenocytes were then removed from the culture dishes and a limiting dilution assay for CTL precursors (CTLp) was performed as previously described [5]. Briefly, replica cultures of splenocyte responder cells were cultured with irradiated (20 Gy) ACI or WF (RT1.A^{u}, third-party) splenocyte stimulator cells. Seven days later, CTL killing of ^{51}Cr-labelled, concanavalin-A stimulated, ACI or third-party WF target cells was determined. CTLp frequency was calculated as described previously [4].

Portal vein injection and liver transplantation

Hepatocytes transfected with either pcRT.45 or pcRQ.B3 were removed from collagen-coated dishes using a trypsin–EDTA solution, and equal numbers of cells transfected with each plasmid were pooled for portal vein injection. A total of 1×10^7 hepatocytes (transfected with 5×10^6 pcRT.45 + 5×10^6 pcRQ.B3 were injected into the portal venous system of Lewis rats via the superior mesenteric vein. Seven days after injection, an ACI orthotopic liver transplantation [9] was performed on injected Lewis rats and animal survival was determined.

Results

In all experiments, the expression of alloantigen was measured in cultures from transfected hepatocytes by an RT1.A^{a}-specific enzyme-linked immunoadsorbant assay (ELISA) [5]. Cell lysates containing membrane-bound antigens from pcRT.45-transfected cells showed the presence of 1500–2800 ng of RT1.A^{a} per culture dish (approximately 1.5×10^6 cells). The amount of soluble RT1.A^{a} in culture supernatants from pcRQ.B3-transfected cells ranged from 1700 to 3000 ng per 1.5×10^6 cultured hepatocytes. All lysate and culture supernatant samples were taken for RT1.Aa measurement 48–72 h post-transfection. We have previously reported similar quantities of membrane-bound or soluble RT1.A^{a} following hepatocyte transfection with the same plasmids [6].

Table 1 Inhibition of cytotoxic T-lymphocyte precursor priming following lymphocyte exposure to autologous hepatocytes simultaneously expressing membrane-bound and secreted allo-MHC antigen (*f* frequency)

Hepatocyte transfection	Experiment 1 specificity: ACI (1/f)	Experiment 2 specificity:	
		ACI (1/f)	WF third-party (1/f)
pCMVLux (control)	2946	7086	11340
pcRT.45	1783	2257	11170
pcRT.45 + pcRQ.B3	6124	6534	11846

To test whether secreted allo-MHC class I antigen could inhibit the CTLp priming effect of allo-MHC membrane-bound antigen, Lewis splenocytes were conditioned for 2 days in cultures of hepatocytes that had been simultaneously transfected with pcRT.45 and pcRQ.B3 (Table 1). In this group of experiments, exposure to hepatocytes expressing only membrane-bound RT1.A^{a} primed CTLp, as evidenced by an increase in CTLp frequency, compared to the control. However, exposure of splenocytes for 2 days to hepatocytes transfected with both pcRT.45 and pcRQ.B3 inhibited the CTLp priming effect of membrane-bound alloantigen, when compared to the CTLp frequency following splenocyte co-culture with only pcRT.45-transfected hepatocytes. It is important to note that the amount of membrane-bound antigen in lysates from hepatocytes transfected with both pcRT.45 and pcRQ.B3 was not less than that measured by ELISA after pcRT.45 transfection only (data not shown). Therefore, the lower CTLp frequency associated with the co-transfection was likely due to soluble alloantigen, and not due simply to the presence of less membrane-bound alloantigen in the cultures. We also tested whether the CTLp effect observed was antigen specific by stimulating similar hepatocyte-conditioned Lewis splenocytes with third-party irradiated splenocytes in the limiting dilution assays. CTL killing of third-party targets was then assessed. CTLp frequency was not affected in these experiments, suggesting that the effect of the different forms of hepatocyte-expressed RT1.A^{a} was antigen specific.

In a previous study we have shown that portal vein injection of as few as 5×10^6 autologous hepatocytes ex-

Table 2 Effect of portal venous injection of autologous hepatocytes expressing membrane-bound and soluble donor MHC class I antigen on liver allograft survival. Note that injection of only pcRT.45-transfected hepatocytes has been previously shown to accelerate ACI liver allograft rejection to 8.4 ± 0.6 days, compared to controls [7]

Transfected hepatocytes injected	Survival time (days)	Statistics (log-rank test)
No injection (control)	10, 11, 11, 11, 11, 12, 12	
pcRT.45 + pcRQ.B3	11, 12, 12, 13	$P = 0.098$ versus control

pressing membrane-bound donor MHC class I antigen accelerates liver allograft rejection by approximately 2–3 days (mean survival = 8.5 ± 0.6 days) [7]. To test whether soluble donor MHC class I antigen could inhibit the in vivo priming effect of hepatocytes expressing membrane-bound donor MHC, the portal venous system of prospective Lewis recipients was co-injected with 5×10^6 pcRT.45-transfected hepatocytes and an equal number of pcRQ.B3-transfected cells, 7 days prior to ACI liver transplantation. Results from these experiments indicated that ACI liver allograft rejection was not accelerated in any of the tested animals in the presence of both membrane-bound and soluble donor MHC antigen (Table 2). Conversely, liver allograft survival was not significantly prolonged after preexposure to both forms of the donor alloantigen.

Discussion

In the present study we examined the possibility that soluble donor allo-MHC antigen can be effective at inhibiting priming by membrane-bound forms of alloantigen. Using our hepatocyte transfection system we were able to provide evidence in vitro that soluble allo-MHC class I antigen is effective at preventing priming of allo-specific CTLp. Inhibition of immunologic priming was also indicated in a liver transplant model, where simultaneous injection of hepatocytes expressing soluble donor MHC class I antigen with hepatocytes expressing membrane-bound alloantigen, abrogated acceleration of liver allograft rejection. It is also interesting to note that the liver allograft survival was not prolonged in the presence of soluble and membrane-bound antigen. Therefore, the soluble antigen prevented priming, but did not prolong liver allograft survival. However, this is consistent with our previous study [6], which indicated that injection of $> 5 \times 10^6$ pcRQ.B3-transfected cells was necessary to prolong liver allograft survival (only 5×10^6 were injected in the present study). Future experiments where higher numbers of pcRQ.B3-transfected cells are injected with pcRT.45-transfected hepatocytes are planned.

The mechanism of the observed in vitro and in vivo effects is not known and was not the focus of this study. However, one possible explanation could relate to the study recently reported by Zavazava and Kroenke [12]. They provide evidence that soluble allo-MHC class I antigen induces expression of CD95 ligand by CTL, which results ultimately in apoptosis of CD95-expressing allo-specific CTL. Consistent with this hypothesis, it is possible that CTL primed by membrane-bound antigen were induced to express high levels of CD95, making them more susceptible to apoptosis via CD95 ligand upregulation by soluble alloantigen. Determining the exact mechanism of this effect will be the subject of further investigation.

Since hepatocytes from a liver transplant likely express both membrane-bound and soluble allo-MHC class I antigens, the immunologic effect(s) of these cells following liver transplantation becomes complex. Data from our present and previous studies [6, 7] suggest that membrane-bound antigen on hepatocytes is quite effective at sensitization of allo-specific CTL in vitro and in vivo. In contrast, soluble alloantigen produced by hepatocytes appears to downregulate CTL under the same experimental conditions. Therefore, since hepatocytes express both forms of the antigen, these data would suggest liver transplantation likely has the potential to be sensitizing and immunosuppressive, at least with regard to CTL activity. Further development of methods to deliver hepatocytes expressing soluble, but not membrane-bound, donor MHC class I antigen may be useful in organ transplantation.

Acknowledgements. This work was supported by the NIH (AI39741-01), the Alabama American Heart Association (AL-G-960046), and the Deutsche Forschungsgemeinschaft (GR1478/2-1).

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