Chimeric Liver Allografts Lack the Ability to Induce Spontaneous Tolerance after Orthotopic Liver Transplantation

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Chimeric Liver Allografts Lack the Ability to Induce Spontaneous Tolerance after Orthotopic Liver Transplantation

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Soluble major histocompatibility complex (MHC) class I antigens released from hepatocytes and the passenger leucocyte (PL) population of the liver allograft have both been considered important contributors for spontaneous liver tolerance. This study was conducted to delineate the role of PLs in more detail. Whereas liver allografts of Dark Agouti (DA) donors were rejected in Lewis graft (LEW) recipients, orthotopic liver transplantation of LEW grafts in DA recipients induced spontaneous tolerance. Irradiated LEW livers where the PL population has been successfully eliminated loose their ability to survive in allogeneic DA hosts, whereas they survive in syngeneic hosts. Reconstitution of irradiated LEW livers with syngeneic (but not with allogeneic) PLs restored tolerance induction. Despite this, tolerance induction liver chimerism was not related to long-term chimerism in DA recipients (spleen, thymus, and blood). Interestingly, PLs of LEW origin can also stimulate graft rejection of irradiated DA livers in syngeneic DA hosts. PLs play an ambiguous role as protectors of liver allografts. This phenomenon is not relying on the induction of micro- or macrochimeric hosts. We speculate that the liver parenchyma determines the dual function of PLs in liver transplantation. This work reveals the significance of PLs as important contributors for spontaneous liver tolerance. Elimination of donor PLs results in graft rejection, whereas reconstitution of these cells restores tolerance induction. Liver tolerance appears to be mainly induced in the graft itself, as this phenomenon is not relying on the induction of micro- or macrochimeric hosts.

ABBREVIATIONS

PL passenger leucocytes
OLT orthotopic liver transplantation

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Introduction

It has long been known that in certain rat strain combinations, orthotopic liver allografts are spontaneously accepted without immunosuppression and induce donor-specific tolerance to further skin and heart grafts in the recipient. The mechanisms of this natural hepatocyte-related tolerance phenomenon have not been completely evaluated in detail. Soluble major histocompatibility complex (MHC) class I antigens released from hepatic parenchyma,[1] microchimerism,[2,3] liver suppressor factors,[4] activation or generation of suppressor T cells,[5] cytokines,[6] and the passenger leucocyte (PL) population[7-9] of the liver allograft are proposed to mediate tolerance induction. This study was conducted to delineate the role of passenger leucocytes in this context in more detail.

Materials and Methods

Arterialized orthotopic liver transplantation (OLT) according to the method described by Engemann et al.[10] was performed in the following

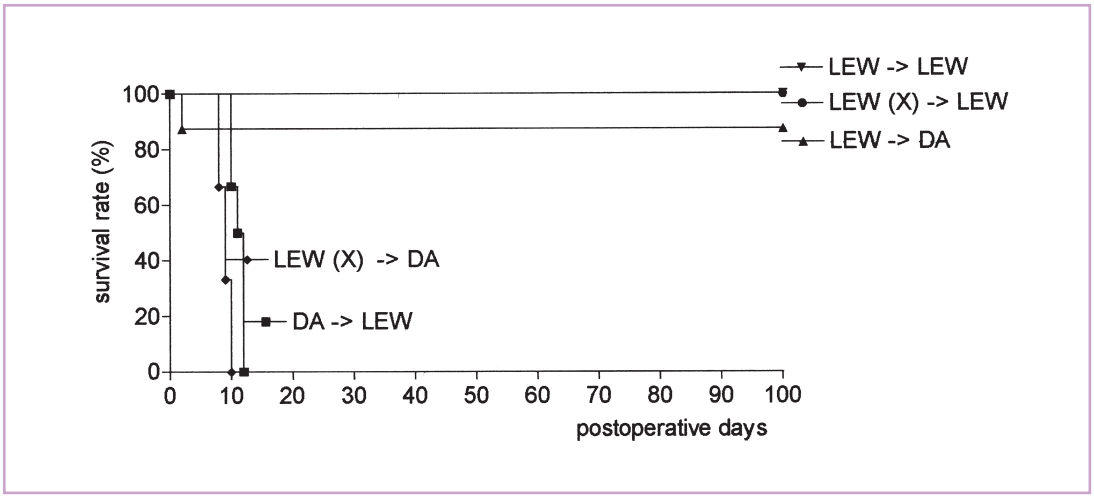

Figure 1. Kaplan Meier blots were generated and compared with log rank (Savage-Mantel-Cox) analysis. Each group consisted of 4 to 6 animals. Liver allografts of Dark Agouti (DA) donors were rejected in Lewis (LEW) recipients 11.2 ± 1.0 days after transplantation. Conversely, orthotopic liver transplantation of LEW grafts in DA recipients induced spontaneous tolerance (organ survival > 100 days). Elimination of donor (LEW) passenger leucocytes by irradiation of the donor prior to transplantation failed to induce tolerance and resulted in an acute rejection of the liver graft after 9.0 ± 0.5 days.

groups (n = 4-6) between male Dark Agouti (DA) [RT1.^{av1}], and Lewis (LEW) [RT1.^{l}], age-matched inbred rat strains:

LEW → LEW,

DA → DA,

DA → LEW,

LEW → DA,

LEW (10 Gy whole body irradiation = WBI, day –7) → LEW,

DA (10 Gy WBI, day –7) → DA,

LEW (10 Gy WBI, day -7) → DA,

LEW (10 Gy WBI, day –7) → LEW (parked for 36 h) → DA,

LEW (10 Gy WBI, day -7) → DA (parked for 36 h) → DA, and

DA (10 Gy WBI, day –7) → LEW (parked for 36 h) → DA.

Immunohistochemical analysis of the liver allografts was performed by alkaline phosphatase antialkaline phosphatase APAAP staining according to the technique described by Cordell et al.[11] using the monoclonal antibodies KiM2R (macrophages), NKR.P-1 3.2.3 (natural killer cells), Ki-S3R (proliferating cells), Ox-3 (LEW MHC class II), and MN4 (DA MHC class I).

Flow cytometric analysis was performed on recipient spleens, and peripheral blood using the monoclonal antibodies was described.

Organ survival rates were calculated according to Kaplan-Meier life-table analysis. Differences in organ survival between the groups were assessed using the generalized Savage (Mantel-Cox) log-rank test (P-value < 0.05).

Results

Whereas liver allografts of DA-donors were rejected in LEW recipients 11.2 ± 1.0 days after transplantation, OLT of LEW grafts in DA recipients induced spontaneous tolerance (organ survival > 100 days). Elimination of donor (LEW) PLs by irradiation of the donor prior to transplantation failed to induce tolerance and resulted in an acute rejection of the liver graft after 9.0 ± 0.5 days (Fig 1). Parking of these irradiated LEW-livers in the allogeneic DA host for 36 h prior to transplantation to the 2nd allogeneic DA recipient was also followed by acute graft rejection (8.0 ± 1.5 days after OLT), although immunohistochemical analysis of these organs revealed an MN4^{+} (DA-specific) population of PLs. Conversely, reconstitution of irradi-

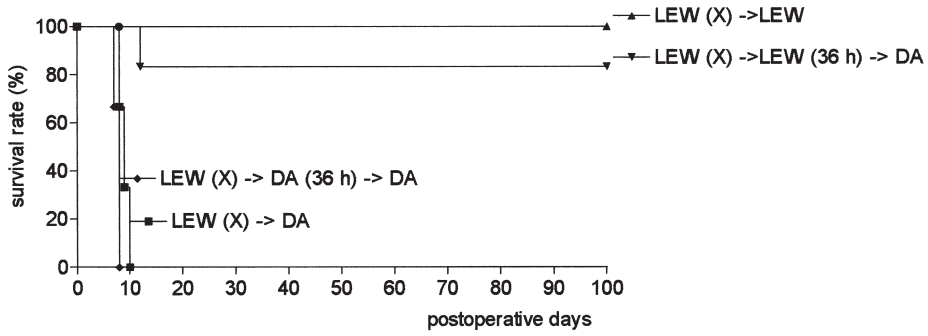

Figure 2. Kaplan Meier blots were generated and compared with log rank (Savage-Mantel-Cox) analysis. Each group consisted of 4 to 6 animals. Parking of irradiated Lewis (LEW)-livers in the allogeneic Dark Agouti (DA) host for 36 h prior to transplantation to the 2nd allogeneic DA recipient was followed by acute graft rejection (8.0 ± 1.5 days after orthotopic liver transplantation). Conversely, reconstitution of irradiated LEW livers with syngeneic passenger leucocytes by parking the irradiated graft in a syngeneic (LEW) recipient restored tolerance induction.

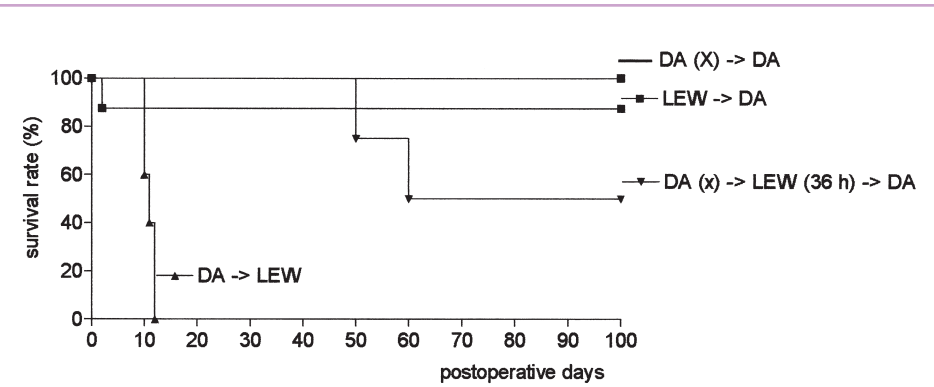

Figure 3. Kaplan Meier blots were generated and compared with log rank (Savage-Mantel-Cox) analysis. Each group consisted of 4 to 6 animals. Passenger leucocytes of Lewis (LEW) origin played an ambiguous role as they were also able to stimulate graft rejection of irradiated Dark Agouti (DA) livers (X) in syngeneic DA hosts. Two of 4 DA recipients that received a syngeneic (DA) irradiated organ parked in a LEW recipient for 36 h revealed acute graft rejection 50 and 60 days posttransplantation.

ated LEW-livers with syngeneic PLs, by parking the irradiated graft in a syngeneic (LEW) recipient, restored tolerance induction (organ survival in 5/6 animals > 100 days) (Fig 2). Immunohistochemical and flow cytometric analysis of donor- and recipient-derived cells revealed chimerism within the graft only, whereas no donor-derived cells could be detected in various recipient organs (thymus, spleen, and peripheral blood). Interestingly, PLs of LEW origin played an ambiguous role as they were also able to stimulate graft rejection of irradiated DA livers in syngeneic DA hosts (Fig 3). Two of 4 DA recipients, which received a syngeneic (DA) irradiated organ parked in a LEW recipient for 36 h, revealed acute graft rejection 50 and 60 days posttransplantation.

Discussion

Chimeric liver allografts consisting of donor (LEW) parenchyma and host (DA) PLs loose their

tolerogeneic capacity. In contrast, syngeneic reconstitution with donor (LEW) PLs restores liver graft acceptance upon transplantation into allogeneic hosts. These results prove the tolerogeneity of PLs, which is sensible to irradiation.[1,12] This phenomenon is not relying upon the induction of micro- or macrochimeric hosts, as no LEW PLs were found in spleen, thymus, or blood compartment from long-term surviving DA rats. For liver transplantants, the "tolerizing" cells are thought to be a special population of liver-resident antigen-presenting cells.[13] Studies of Anderson and Matzinger[13] revealed that chimerism after skin transplantation in mice was not multilineage, but limited to T cells. Immunologic characteristics of these cells indicated that they probably came from a few circulating T cells that happened to be passing through the skin at the time of excision. Interestingly, our data suggest that PLs can both induce tolerance and stimulate rejection. Furthermore, the ambiguous role of PLs also depends on the host's immunological maturity, as well as on the antigeneic disparities between the solid organ transplant and the transferred PLs.[13] This opposite outcome should be considered for further clinical protocols based on chimerism to induce tolerance.

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