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Comparative analysis of the fate of donor dendritic cells and B cells and their influence on alloreactive T cell responses under tacrolimus immunosuppression

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Abstract

We have shown that tacrolimus (TAC)-induced liver allograft acceptance is associated with migration and persistence of donor B cells and dendritic cells (DC). To clarify whether these MHC class II^+ leukocytes have favorable roles in inducing tolerance, we analyzed recipient T cell reactions after allogeneic B or DC infusion. LEW rat B cells localized exclusively in BN host B cell follicles without any direct contact with host T cells. While few donor DC migrated to T cell areas and marginal zones, they were captured by host APC, suggesting that allogeneic MHC class II^+ cells may induce immune reactions via the indirect pathway. Although DC-infused non-immunosuppressed recipients showed enhanced ex vivo anti-donor responses, persistent in vitro donor-specific hyporeactivity was seen equally with donor DC or B cell infusion under TAC. The results indicate that donor MHC class II^+ APC are capable of regulating recipient immune reactions under TAC. Possible involvement of the indirect pathway of allorecognition is discussed.

Keywords: Donor dendritic cells; B cells; Tacrolimus

Introduction

Interstitial bone marrow (BM)-derived "passenger" leukocytes in solid organs migrate to recipient lymphoid tissues after transplantation and are believed to initiate alloimmune reactions leading to acute rejection [1,2]. However, compelling evidence has accumulated that passenger leukocytes may also regulate immune reactions and induce transplant tolerance [3–6]. Donor leukocyte elimination from liver allografts by irradiation before transplantation results in rejection of grafts that would otherwise be accepted without immunosuppression [3]. Similarly, passenger leukocyte depletion in cardiac transplantation precludes the induction of tolerance and results in accelerated acute/chronic rejection [4,5]. Thus, the migration of passenger leukocytes to recipient lymphoid tissues and the early interactions between these antigen-presenting cells (APC) and recipient leukocytes appear to be crucial in directing host immune reactions towards allograft rejection or acceptance.

Although the immunostimulatory roles of passenger leukocytes and the mechanisms involved in host T cell

Abbreviations: APC, antigen-presenting cells; BM, bone marrow; BN, Brown Norway; CMTMR, 5-(and-6)-(((4-chloromethyl)benzoyl)amino)tetramethylrhodamine; CR, chronic rejection; DC, dendritic cells; FCS, fetal calf serum; LEW, Lewis; mAb, monoclonal antibody; PALS, periarteriolar lymphoid sheath; TAC, tacrolimus.

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activation have been well-established, it remains unclear how passenger leukocytes regulate the host immune system in a tolerogenic manner. In some experimental models, donor leukocytes induce host T cell activation and subsequent deletion via activation-induced cell death, leading to tolerance [7,8]. Alternatively, specific populations of passenger leukocytes (e.g., immature dendritic cells [DC]) may tolerize T cells or induce regulatory T cell populations depending on their maturation status and lineage commitment [9,10].

We have reported that in rats, a short course of tacrolimus (TAC) administration induced permanent liver allograft acceptance without histopathological evidence of chronic rejection (CR). Subsequent donor strain challenge cardiac allografts are also accepted by liver allograft-bearing recipients, without treatment and without CR [11,12]. In our previous studies, TAC-induced CR-free liver allograft acceptance was associated with an early intense migration of donor MHC class II^+ B cells and DC that functioned as APC, followed by an increased proliferation and apoptosis of host lymphocytes [13]. In addition, donor MHC class II^+ hematolymphoid cells were maintained in long-term functioning CR-free allografts [12]. These results suggested a role for donor APC in inducing/maintaining tolerance rather than rejection in our TAC-based immunosuppression model.

To further clarify the roles of donor B cells and DC in the induction of tolerance under TAC immunosuppression, we investigated cell migration patterns, interactions with recipient leukocytes, and the resulting T cell responses after infusion of allogeneic B cells or DC. The results demonstrate that allogeneic B cells and DC migrate to distinct B and T cell areas of host lymphoid tissues, respectively. In the absence of immunosuppression, DC were more potent than B cells in priming the host immune system. However, under TAC immunosuppression, both cell types induced alloantigen-specific T cell hyporesponsiveness.

Materials and methods

Animals

Inbred 8–12-week-old male Lewis (LEW, $RT1^l$) and Brown Norway (BN, RT^n) rats were maintained in a pathogen-free laminar flow facility at the University of Pittsburgh. All experimental procedures were performed in accordance with the guidelines of the Council on Animal Care at the University of Pittsburgh.

Organ and cell transplantation

LEW heterotopic heart transplantation was performed into BN recipients [11]. Heart graft survival was monitored by trans-abdominal palpation, and rejection was defined by the cessation of heartbeat, followed by histopathological confirmation. DC and B cells (10×10^6 to 20×10^6 per animal) were obtained from normal LEW and injected intravenously (i.v.) into BN recipients via the penile vein.

Immunosuppression

TAC (Fujisawa Pharmaceutical Co., Osaka, Japan) in normal saline was injected intramuscularly (0.5–1.0 mg kg^{-1} day^{-1}), depending on experiments. These doses of TAC have been shown to induce long-term (>100 days) rat organ allograft survival [11].

Preparation of lymphocyte subsets from spleen and lymph nodes

Single-cell suspensions were made from cervical and mesenteric lymph nodes and spleens. T cells were isolated by negative selection using T cell enrichment columns (R&D Systems, Inc., Minneapolis, MN) with purity >95%. B cells were obtained by positive selection using anti-rat Ig immunobead columns (Biotecx Laboratories Inc., Houston, TX) after removing the adherent and phagocytotic cells with a purity of >85%. Flow cytometric analysis of the purified B cell fraction showed no $ED2^+$ macrophages or $CD11c^+$ DC. The majority of non-B cells in the fraction were T cells.

BM-derived DC propagation

DC were propagated from normal LEW or BN BM as described with modifications [14,15]. BM cells were isolated from the femurs and tibias by flushing with RPMI-1640 containing 10% v/v FCS (Life Technologies, Grand Island, NY). BM cells were cultured ($2–3 \times 10^6$/ml) in complete RPMI-1640 containing 10% v/v FCS, 25 mM HEPES buffer, 5×10^{-5} M 2-ME, 2 mM L-glutamine, penicillin (50 U/ml), and streptomycin (50 μg/ml) in the presence of 1000–1500 U/ml mouse rGM-CSF (Amgen, Seattle, WA), 5 ng/ml rat rIL-4 (R&D Systems, Inc.) and 25–50 ng/ml mouse rFlt3L (R&D Systems, Inc.). Cultures were fed every 2–3 days by aspirating 50% of the culture supernatant and replacing it with fresh media containing cytokines. After 7–10 days at 37°C in 5% CO_2, non-adherent cells and loosely adherent cell aggregates were recovered by gentle pipetting and centrifugation. The phenotype and allostimulatory function of the propagated DC were analyzed by flow cytometry and MLR, respectively.

Monoclonal antibodies

Monoclonal antibodies (mAbs) used in this study included OX6 (MHC class II), 8A2 (CD11c), 3H5 (CD80), 24F (CD86), 1A29 (ICAM-1; CD54), ED1, ED2 and ED3 (macrophages), R7.3 (αβTCR), OX33 (CD45RA;