Transpl Int (1994) 7 [Suppl 1]: S559–S562

Charles W. Hewitt
Ramon Llull
Mayuri P. Patel
Kenneth R. Beko II
Kirby S. Black
Donald C. Martin

Mechanisms of unresponsiveness associated with pretransplant blood transfusion – cyclosporine-induced mixed lymphocyte chimerism

C. W. Hewitt (✉) · R. Llull · M. P. Patel
K. R. Beko II · K. S. Black · D. C. Martin
Division of Surgical Research,
Department of Surgery,
UMDNJ-Robert Wood Johnson Medical School, Cooper Hospital,
3 Cooper Plaza Suite 411,
Camden, NJ 08103, USA

University of Pittsburgh, Pittsburgh, PA
and University of California, Irvine, USA

Supported in part by grants from The National Institute of Health (no. DK-36332-03); The International Association of Fire Fighters Burn Foundation; The Muscular Dystrophy Association; Sandoz Ltd; EPIX Pharmaceutical Inc.; The American Association of Plastic Surgeons; an American Society for Hand Surgery Fellowship; a Scholarship of Savings Bank 'Sa Nostra' of the Balearic Islands, Spain; a 1991 scholarship and grant of the US Fullbright Commission and the Ministry of Education and Science, Spain; a 1992 fellowship of the Plastic Surgery Educational Foundation; and The National Kidney Foundation

Abstract Multiple pretransplant blood transfusions while under limited daily cyclosporine cover (PTBT–CsA) induce extensive rat renal allograft survival and antigen-specific non-responsiveness. The underlying mechanisms of this extensive allograft survival are not yet fully understood. We hypothesized that one of the potential contributing mechanisms to tolerance induction in PTBT–CsA-treated kidney recipients is the development of stable mixed chimerism, putatively due to the proliferation of stem cells capable of haematopoiesis in the transfused blood. BN rats served as whole blood and kidney donors. LEW rats served as recipients of the PTBT–CsA protocol and BN kidney transplants. Three weekly transfusions were given under concomitant limited CsA cover. Following these multiple primary sensitizations, antigen-specific splenic cellular responsiveness in vivo was normal in comparison with naive animals. However, these experimental splenocytes were non-specifically suppressed against third-party allodeterminants. At 100 days post-transplantation (T_{100}) following tolerance induction to kidney allografts (secondary challenge), in vivo adoptive transfer experiments demonstrated the existence of potent splenic suppressor cells. In vitro suppressor cell assays confirmed that these cells were non-specific suppressor cells. However, following chimerism stabilization at T_{130}, splenic antigen-specific suppressor cells became exclusively expressed in the tolerant animals, replacing the non-specific suppressor cells. At this time, splenic microchimerism was at peak levels and remained stable from T_{100} to T_{130}. In conclusion, these findings demonstrate that sequential mechanisms of suppressor cell network expression are induced within a chimeric environment by blood-CsA immune modulation. Stable mixed lymphocyte chimerism and related immunomodulatory mechanisms may, therefore, play an important tolerogenic role in blood-CsA-induced non-responsiveness and in the beneficial effect of blood transfusion.

Key words Blood transfusion
Immunosuppression · Tolerance
Chimerism · Cyclosporine

Introduction

Multiple pretransplant blood transfusions while under limited daily cyclosporine cover (PTBT–CsA) induce indefinite rat renal allograft survival and antigen-specific non-responsiveness [1]. We have previously reported data suggesting that a donor-derived cell population is required for the beneficial effect of donor-specific blood transfusions to occur in kidney transplantation [2, 3]. Progenitor immunocompetent cells capable of haematopoiesis are known to exist in the blood stream [4]. Recently, mechanisms of PTBT-induced specific non-responsiveness in relation to the induction of mixed lymphocyte chimerism and tolerance have been debated [5, 6]. We hypothesized that, under appropriate immunosuppression, progenitor stem and immunocompetent cells would be transferred by blood transfusion, proliferate within privileged immune tissues such as the spleen, and give rise to a chimeric immune system. This would be a contributing mechanism to the immunosuppressive effect of blood transfusion. Our hypothesis is compatible with data demonstrating that spleens and lymph nodes from mice also develop chimerism under similar experimental conditions [7].

Materials and methods

LEW rats served as recipients of BN kidney allografts and donor-specific blood transfusions. The PTBT–CsA protocol consisted of a separate 1 ml transfusion of whole blood given to each recipient at −3 and 2 weeks and 1 week prior to transplantation. Recipients received prior concomitant CsA cover (subcutaneous, 5 mg/kg per day) from day 28 to day 5 prior to kidney transplantation. This PTBT–CsA protocol induces permanent renal allograft survival in this genetic combination [8]. Ten long-term LEW recipients of BN renal allografts were studied beyond 100 days post-transplantation. In vitro assays were performed at the time of transplantation (T_0) and at 100 and 130 days post-transplantation (T_{100}, T_{130}), when development of splenic chimerism was observed. Levels of CsA were known to be undetectable at these time-points.

Recipients underwent bilateral nephrectomy [8]. Grafting involved anastomosis of the donor renal artery to the recipient abdominal aorta, the donor renal vein to the recipient inferior vena cava and a donor bladder patch to the recipient bladder. Animals were observed over the following 7 days for urinary output. Renal function during the course of recipient survival was assessed by blood and urea nitrogen levels at weekly intervals. T-cell-enriched populations were isolated from spleen and peripheral blood by gradient centifugation. Red blood cells were removed by hypotonic shock and B cells (Ig^+) were depleted by incubation with anti-rat IgG(Fc)-coated magnetic beads. Quantitation of T-cell chimerism was determined by experimental lymphocyte labelling of donor-derived (BN^+) populations with polyclonal LEW anti-BN rat alloserum, flow cytometry, regression analysis of known chimeric populations, and inverse prediction. For in vivo evaluation of lymphocyte responsiveness, one-way popliteal lymph-node reactivity was evaluated following foot-pad inoculation of experimental and control splenocytes. Following background substration, the ratio of popliteal lymph-node reactivity between maximal and experimental responses was quantitated (mean stimulation index, MSI). Experimental and control splenocytes were adoptively transferred for in vivo suppressor cell studies. In vitro suppressor assays were done by coculturing chimeric cells in naive mixed lymphocyte reactions.

Results and discussion

The percentage of BN^+ splenic cells over sequential time-points are depicted in the top panel of Fig. 1. At T_0 levels of BN^+ chimeric donor T-cells were hardly detectable in the spleen ($1.7\% \pm 0.9\%$, Fig. 1). Yet at T_{100} the level of BN^+ T-cell chimerism increased to $10.1\% \pm 3.8\%$. At T_{130} the percentage of BN^+ T cells remained unchanged with respect to chimerism levels at T_{100} ($8.9\% \pm 5.4\%$, Student's t-test P values not significant). The peripheral immune compartment, however, failed to demonstrate levels of mixed allogeneic T-cell chimerism (data not shown). It was thought that these levels of splenic chimerims might develop graft versus host disease (GVHD). However, clinical and histopathological evidence of GVHD was not observed.

Immediately after transplantation (T_0), when levels of splenic chimerism were not detectable, in vivo one-way mixed lymphocyte reactivity was evaluated. The central panel of Fig. 1 shows in vivo reactivity as percent suppression with respect to maximal response at T_{28} when no chimerism was present. These results indicate normal antigen-specific responsiveness at T_0 (MSI experimental 5.8 ± 1.6, control 5.7 ± 1.9; P value not significant). However, when BN^+ splenic T cells reached 10.1% at T_{100}, anti-BN alloresponses were found to be significantly suppressed compared with both the control and the T_0 values ($P < 0.05$). Also, alloantigen non-specific cellular suppressive mechanisms were effective at this time within the chimeric spleen against irrelevant (ACI) allodeterminants. In contrast, in vivo non-specific (anti-ACI) cellular responses were suppressed at T_0 (MSI experimental 6.3 ± 4.9, control 13.4 ± 2.8; $P < 0.05$) when no detectable chimerism was found. These cellular responses did not significantly change during the post-operative period of chimerism development (6.3 ± 4.9 at T_0, 5.7 ± 3.7 at T_{100}, P values not significant). These in vivo results suggest that BN^+ cells were not required for this non-specific suppression to develop.

It was hypothesized that immunosuppression at T_{100} may be attributed to the development of non-specific suppressor cell networks. Suppressor cells capable of modulating donor-specific allogeneic responses were

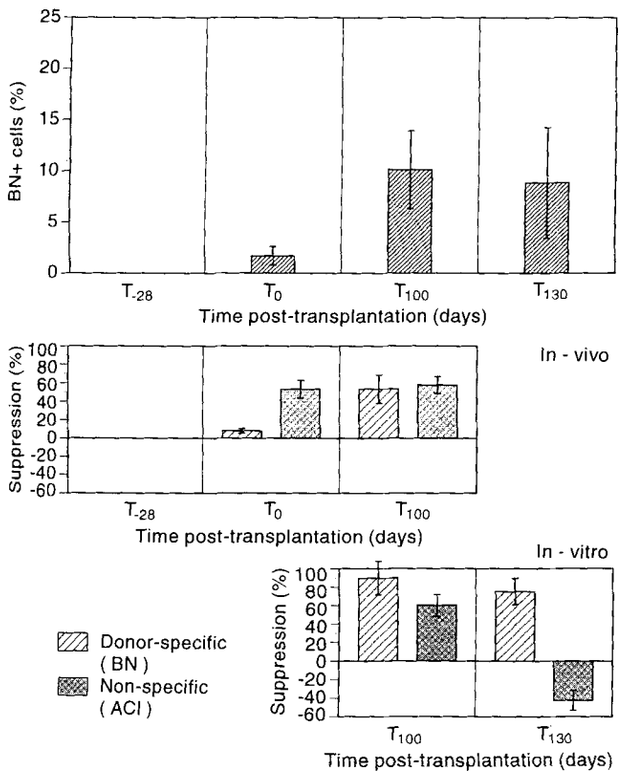

Fig. 1 Levels of BN^+ chimeric cells over time as related to concomitant in vivo unresponsiveness and in vitro expression of non-specific and antigen-specific suppressor cells. Error bars represent standard deviation. The *top panel* represents the percentage of chimeric cells at different time-points (T_{28}, T_0, T_{100} and T_{130}). The *central panel* represents in vivo mixed lymphocyte responses at −28, 0 and 100 days post-transplantation. Mean stimulation index is represented as percent suppression with respect to maximal responses (percent suppression of mean stimulation index = (1 − experimental response/maximal response) × 100, following substraction of appropriate backgrounds). In vitro coculture suppressor assays of mixed lymphocyte reactions are represented in the *bottom panel*. Percent suppression was calculated

shown by significant graft prolongation in secondary LEW recipients of experimental splenocytes and BN renal allografts ($P < 0.01$). Coculture of these chimeric suppressor cells was undertaken to test the ability of the splenic chimeric cells to suppress donor-specific and third-party-activated mixed lymphocyte reactions of LEW naive splenocytes (Fig. 1, bottom panel). The splenic chimeric immunocytes were characterized as potent non-specific suppressor cells by their ability to inhibit various cellular immune responses. Further investigations were carried out to determine whether these splenic non-specific suppressor circuits remained effective past 100 days and following secondary challenge with donor-specific skin grafts. These grafts had undergone antigen-specific prolongation and defined operational tolerance. Surprisingly, following this tertiary alloimmune challenge, alloantigen-activated suppressor cell assays demonstrated that only antigen-specific suppressor cell circuits were now active at T_{130} ($P < 0.001$).

In conclusion, we found convincing evidence in these PTBT–CsA-tolerant animals for the development of low-level mixed allogeneic immune chimerism. The expansion of splenic chimerism induced by PTBT–CsA enhancement is associated with development and maturation of suppressor cell networks. Non-specific suppressor cells, which have been ascribed to natural suppressor cells [9], are known to occur in association with haemopoiesis, GVHD and tolerance. Consistent with these findings, non-specific suppressor cells were identified during the early phases of tolerance induction [10]. Interestingly, however, following tolerance induction and tertiary alloantigen challenge past 100 days, non-specific suppressor cells were replaced by antigen-specific suppressor cells in the spleen at a time when donor T-cell chimerism was maximal. Further studies are underway to investigate the role of stable immune T-cell chimerism in suppressor cell circuit expression and tolerance induction. Stable mixed alloimmune chimerism may represent an important mechanism in blood-CsA induced tolerance and the immunosuppressive blood transfusion effect [11].

References

1. Hewitt CW, Black KS, Harman JC, et al (1990) Partial tolerance in rat renal allograft recipients following multiple blood transfusions and concomitant cyclosporine. Transplantation 49:194–198
2. Beko KR II, Tran HO, Hewitt CW, et al (1991) Mechanisms of prior blood transfusion-cyclosporine-induced tolerance: A potential role for immune-cellular chimerism. Transplant Proc 22:147–148
3. Martin DC, Hewitt CW, Osborne J, et al (1982) Enhanced kidney graft survival in rats by single or multiple blood transfusion(s) and various blood products. Transplant Proc 14:407–409

4. Stiff PJ, Koester AR, Eagleton LE, Hindman T, Braud E, Wiedner MK (1987) Autologous stem cell transplantation using peripheral blood stem cells. Transplantation 44:680
5. de Waal LP, van Twuyver E (1991) Blood transfusion and allograft survival. Is mixed chimerism the solution for tolerance induction in clinical transplantation? Crit Rev Immunol 10:417
6. van Twuyver E, Mooijaart RJ, ten Berge IJM, et al (1991) Pretransplantation blood transfusion revised. N Engl J Med 325:1210–1213
7. Heeg K, Wagner H (1990) Induction of peripheral tolerance to class I major histocompatibility complex (MHC) alloantigens in adult mice: transfused class I MHC-incompatible splenocytes veto clonal responses of antigen-reactive Lyt-2^+ T cells. J Exp Med 172:719–728
8. Martin D, Hewitt CW, Black KS, Dowdy SF, Quintero CS, Reyes M Jr (1987) Extensive prolongation of rat renal allograft survival following donor or non-specific transfusions and concomitant immunosuppressant. Transplantation 43:790
9. Stober S (1984) Natural suppressor (NS) cells, neonatal tolerance, and total lymphoid irradiation: Exploring obscure relationships. Ann Rev Immunol 2:219
10. Maier T, Holda J, Claman H (1986) Natural suppressor (NS) cells. Members of the LGL regulatory family. Immunol Today 7:312
11. Sykes M, Sharabi Y, Sachs D II (1990) Natural suppressor cells in spleens of irradiated, bone marrow-reconstituted mice and normal bone marrow: Lack of Sca-1 expression and enrichment by depletion of Mac1-positive cells. Cell Immunol 127:260–274