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BRIEF REPORT

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Double bone marrow transplantation for severe aplastic anemia after orthotopic liver transplantation: implications for clinical management and immune tolerance

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Abstract A 2-year-old boy underwent liver transplantation for fulminant hepatic failure of unknown cause. Four months later the child developed severe aplastic anemia. Allogeneic bone marrow transplantation (BMT) was performed using marrow from his 14-month-old HLA-identical sister. Severe aplastic anemia recurred 2.5 months later. After reconditioning a second BMT was performed using the same donor. Tapering of immunosuppression 2 years after BMT led to biopsy-confirmed rejection of the liver. Therapy with high-dose corticosteroids and an increase in cyclosporine A medication readily reversed rejection and a low-dose immunosuppression reflected by cyclosporine trough levels less than 50 ng/ml has been maintained since. Eight years later the boy is in excellent health with both bone marrow and liver functioning perfectly. In summary, this case demonstrates that even recurrent severe aplastic anemia after OLT can be cured by BMT, and that a transplanted liver can tolerate a double conditioning regimen without problems. Tolerance towards the liver through BMT did not develop.

Keywords Double bone marrow transplantation · Orthotopic liver transplantation · Childhood · Clinical managementTolerance

Introduction

Bone marrow transplantation (BMT) following solid organ transplantation in childhood is rare, with only two cases reported in the literature [9, 22]. This situation presents a challenge in clinical management and at the same time an opportunity to learn about immune tolerance.

Chemotherapy must be given in preparation for BMT to prevent rejection and to create space for the new cells. It has not been established whether a transplanted organ, especially the liver, can tolerate the dose of cytoreductive therapy needed for BMT. Furthermore, it is unclear whether cytoreductive therapy can be repeated in the case of bone marrow (BM) failure. There are no guidelines when to discontinue the immunosuppressant during conditioning, and whether to switch the immunosuppressant since they often differ in liver transplantation (tacrolimus) and BMT (cyclosporine). Viral reactivation often leads to dangerous complications since high-dose immunosuppressive therapy must be maintained for several months. Furthermore, it is not

known whether a transplanted liver which is nearly always HLA incompatible and sometimes also AB0 incompatible is at an increased risk of immune-mediated attack by the transplanted cells, or whether a state of tolerance can be achieved after BMT. Attempts to achieve tolerance towards the solid organ have recently been made by cotransplantation of BM cells from the same donor. The resulting microchimerism is reported to induce immune modulation, prolong graft survival, and sometimes lead to tolerance [6, 19]. An interesting situation arises when a new immune system is transferred by an HLA-identical marrow transplantation into a patient with an HLA-different transplanted solid organ already present. The immune system must be educated in the presence of the graft's HLA antigens. This could lead to the acceptance of the organ graft. On the other hand, immune stimulation by foreign antigens may lead to increased graft-versus-host disease or to fulminant rejection.

Case report

A 2-year-old boy was admitted to our center in November 1991 with clinical and biochemical signs of fulminant hepatic failure (FHF). An upper respiratory tract infection 3 weeks earlier led to an icteric picture with vomiting and acholic stools. The patient was in good clinical health until hospital admission. Within hours the boy developed grade III hepatic encephalopathy. Intense diagnostic workup did not reveal the cause of FHF. Histology of the liver showed unspecific fulminant hepatitis. IgG but not IgM antibodies were found against hepatitis A virus, cytomegalovirus (CMV), and Epstein- Barr virus. Excretion of CMV was noted in urine, saliva, and bronchial secretions from 7 to 11 November 1991. An emergency AB0-mismatched orthotopic liver transplantation (OLT) from a male donor was performed at Saint Luc Hospital in Brussels, Belgium, 2 days after admission in our hospital. Cyclosporine A (CsA) and prednisolone were used as initial immunosuppression. In the early post-transplantation period acute allograft rejection confirmed by liver biopsy occurred that could be reversed with high-dose corticosteroids and OKT3 in addition to CsA. Two weeks later a second liver biopsy showed positive immunohistology for CMV but no evidence of rejection. Leukopenia [white blood cell count (WBC) 600/μl] and thrombocytopenia (12,000/μl) developed. Ganciclovir and multiple transfusions were given.

One week later a third biopsy showed severe rejection and persistence of CMV hepatitis. Again, OKT3 and corticosteroids were given, and immunosuppression was switched from CsA to tacrolimus (FK506). There was rapid normalization of liver function test (LFT) findings, but leukopenia remained a central problem despite 14 days of granulocyte/macrophage colony stimulating factor (GM-CSF) treatment. One month later the patient presented with mechanical ileus and peritonitis due to perforation of a Meckel's diverticulum which had to be treated surgically. Hematological values were normal until March 1992, when severe leukopenia (WBC 800/μl, 98% lymphocytes) and thrombocytopenia (10,000/μl) occurred. LFT findings were normal. Bone marrow (BM) aspiration and BM biopsy showed a severely aplastic marrow with no megakaryocytes and very low granulopoiesis, consistent with the diagnosis of very severe aplastic anemia. Therapy with GM-CSF (250 μg/m^2 daily), then granulocyte colony-stimulating factor (G-CSF) in increasing doses (5, 10, 20 μg/kg body weight daily), and finally a combination of GM-CSF (250 μg/m^2 daily) and G-CSF (20 μg/kg daily) was given. As there was still no improvement, BMT from the patient's 14-month-old HLA-identical, MLC-negative, and blood group identical sister was planned. After conditioning with antilymphocyte serum of horse origin (10 mg/kg daily on days −4, −3, −2; Lymphoglobulin Merieux, Institut Merieux Transplant, Leimen, Germany) and cyclophosphamide (50 mg/kg daily, on days −5, −4, −3, −2) a total of 6.5×10^8/kg nucleated marrow cells were transfused on 28 April 1992. FK506 was discontinued on day −5. CsA (3 mg/kg intravenously) was started on day −1, together with methotrexate given intravenously on days +1, +3, +6, +11.

No major problems occurred. LFT findings remained normal, with no signs of veno-occlusive disease. Engraftment was rapid. The last platelet concentrate was given on day +9. Platelets exceeded 100,000/μl on day +24, WBC exceeded 1,000/μl on day +20 and 2,000/μl on day +32. More than 0.5% reticulocytes were seen constantly after day +18. The boy was discharged on day +38 in excellent condition and with normal blood counts. CsA polyclonal levels were maintained around 380 ng/ml (therapeutic range 200–400 ng/ml) with normal blood counts.

On day +72 a sharp drop in leukocytes (to 1,700/μl) and a moderate drop in platelets (162,000/μl) were noted. BM aspiration showed hypocellularity with mast cells and all stages of progenitors present. In situ hybridization for X- and Y-chromosomes showed the presence of 55% male (recipient) BM cells. IgM reactivity for adenovirus was found. CsA had to be tapered to allow an immune response against the viral infection. However, the proportion of recipient BM cells increased to 70% on day +90. Assuming an ongoing rejection, CsA was increased again and corticosteroids were started on day +92. Concurrently a positive CMV early antigen was found in the boy's blood on day +87. Despite therapy with ganciclovir for 1 week, intravenous immunoglobulin transfusions and G-CSF in increasing doses (5 μg/kg daily, then 10 μg/kg daily) for 3 weeks leukopenia and thrombopenia persisted. The BM was extremely aplastic, and for the first time also lymphocytic infiltrates were seen, consistent with the diagnosis of rejection.

A second BMT from the same donor was performed 126 days after the first BMT and 10 months after OLT. The conditioning regimen consisted of total nodal lymphoid irradiation (250 cGy daily, days −7, −6, −5), rabbit antithymocyte globulin (ATG-Fresenius, Fresenius, Bad Homburg, Germany; 5 mg/kg/ daily, days −4, −3, −2), and cyclophosphamide (50 mg/kg intravenously per day, days −5, −4, −3, −2). A total of 9.7×10^8/kg nucleated cells were given on 9 January 1992. CsA was stopped on day −5 and restarted (3 mg/kg intravenously per day) on day −1; methotrexate was given on days +1, +3, +6, +11. Again, no major problems were observed. Liver function remained normal, with only serum glutamic pyruvic transaminase moderately increased to a maximum of 35 U/l (1.5-fold the upper normal level) while both glutamic oxaloacetic transaminase and γ-glutamyltranspeptidase remained within the normal range. No infectious complications occurred. All surveillance cultures for CMV early antigen in blood and urine were negative. Intravenous immunoglobulins were given for antiviral prophylaxis every 2 weeks and acyclovir 500 mg/m^2 intravenously every 8 h.

Engraftment occurred rapidly. The last platelet concentrate was given on day +16. WBC exceeded 1,000/μl on day +17 and 2,000/μl on day +22. More than 0.5% reticulocytes were seen constantly after day +22. The boy was discharged on day +32. Polyclonal CsA levels were maintained between 300 and 350 ng/ml. WBC and platelets were 4,500–6,000/μl and 200,000–320,000/μl, respectively. BM aspiration on day +66 showed complete chimerism and good cellularity. Regular follow-up examinations showed excellent health, normal blood counts, and complete chimerism in the peripheral blood. In 1993 and 1994 mitogen stimulation showed a

reduced response. In the following years a normal mitogen response was observed, with a slightly weaker response to the B cell mitogens pokeweed mitogen and SAC. Antigen response was absent in 1993, reduced in 1994, and normal thereafter. T cell phenotyping showed initially increased expression of CD45RO and HLA-DR on T cells and a predominance of CD8 lymphocytes, which was gradually normalizing. Since only very low immunosuppression was needed over a long period without increased LFT values, and since there are reports of the possibility of tolerance towards the solid organ transplant, we reduced the immunosuppression under strict monitoring of clinical and laboratory parameters.

For more than 2 years the patient received CsA (5.4 mg/kg body weight orally per day), which resulted in a monoclonal CsA blood level of 76 ng/ml (171 ng/ml polyclonal). Beginning in April 1994 immunosuppression was tapered gradually (Fig. 1). A slight increase in glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels appeared with no other laboratory abnormalities. The boy was in perfect clinical health all the time. We performed a percutaneous liver biopsy which showed mild to moderate acute allograft rejection without evidence of toxic organ damage. Rescue therapy with high-dose corticosteroids (14 mg/kg prednisolone intravenously per day for 3 days) was initiated. CsA was increased to 6 mg/kg body weight orally per day, resulting in a polyclonal level of 167 ng/ml and a monoclonal level of 71 ng/ml. Serum transaminases normalized within 2 weeks. Blood counts stayed normal and LFT values remained normal thereafter. No infectious complications, especially no recurrence of CMV, no graft-versus-host disease, and no signs of rejection or toxicity were seen. CsA was tapered without problems to a daily oral dose of 4 mg/kg, with a resulting monoclonal level of 36 ng/ml (polyclonal level 84 ng/ml). The patient is now, more than 8 years after his second BMT, still in excellent health with normal liver function and blood counts.

Discussion

BMT after solid organ transplantation in children has rarely been reported in the literature. A recent survey identified only seven patients given a BMT after solid organ transplant, with four adult patients after kidney transplant and three patients after OLT [4]. Two of the three OLT patients were children [9, 22]. No patient with double BMT after OLT has yet been reported. Possible reasons are that this combination is not common, that the patients are too ill for this double procedure to be performed, or, if performed, that there was no success and the case was therefore not reported. Our case, however, shows that BMT and even retransplantation can be carried out safely after OLT.

Only little is known about whether a transplanted liver can tolerate repeated cytotoxic damage and irradiation. In our case the liver tolerated two conditioning regimens within 4 months without any problems. Even irradiation with 7.5 Gy in the second conditioning regimen did not lead to a significant increase in LFT values or veno-occlusive disease. Another case report [7] showed that a child having received a heart-lung transplantation tolerated chemotherapy for B cell lymphoma without significant organ damage.

FK506 (tacrolimus) is primarily used for solid organ transplantation, while CsA is most frequently given for stem cell transplantation [1]. When stem cell transplantation is performed in a recipient of a solid organ, it is not known whether the primary immunosuppressant should be continued or switched to CsA. Additionally, the time when to switch, and whether it is dangerous to discontinue the immunosuppressant during the conditioning regimen is unknown. In our case FK 506 was discontinued with the beginning of the cytoreductive therapy for the first BMT. No signs of rejection were observed. It appears that during the conditioning regimen no additional immunosuppression is needed. We restarted immunosuppression on day −1 using CsA. CsA was chosen because of the then limited experience with FK 506 in BMT. CsA and FK506 share the same mode of action with similar but not identical adverse effects. FK 506 is rarely used in BMT as first line graft-versus-host disease prophylaxis. It is more often given as a potent rescue immunosuppressant in the case of CsA or steroid resistant graft-versus-host disease [8]. Nowadays, with growing evidence that FK506 can be used safely after BMT [5, 15, 18], it appears equally possible to continue FK 506 after the end of the cytoreductive therapy. This approach has been reported in only a single pediatric patient [22].

An incidence of up to 33% of severe aplastic anemia after OLT for non-A/non-B hepatitis is reported in the literature [2, 16, 23]. Aplastic anemia can be caused by viruses, especially non-A/non-B hepatitis, CMV, and parvovirus B19, drugs, and immune attack. Especially parvovirus B19 has been associated with both fulminant hepatic failure and aplastic anemia [10, 16]. Our patient, however, was found to be negative for anti-parvovirus antibodies. Alternatively, lymphocytes transfused with the donor's liver may have induced the severe aplastic anemia by attacking hematopoietic stem cells. It has been reported that blood donor's lymphocytes can expand in immunocompetent recipients [11]. Comenzo and colleagues [3] found donor lymphocytes 100 days after OLT in the peripheral blood and BM. An infant with a split liver transplanted from her mother developed graft-versus-host disease and aplastic anemia with donor lymphocytes detectable in the blood [24].

There have been reports of tolerance against the transplanted liver after OLT in mice [14], primates [21], and humans [12, 17]. Several studies in humans show enhanced allograft survival [19, 20] when repeated BM infusions are given after allograft transplantation or at the time of OLT. However, these were donor-matched lymphocytes, not third-party lymphocytes as in our case. An interesting question is therefore whether tolerance to a solid organ will develop when a BM

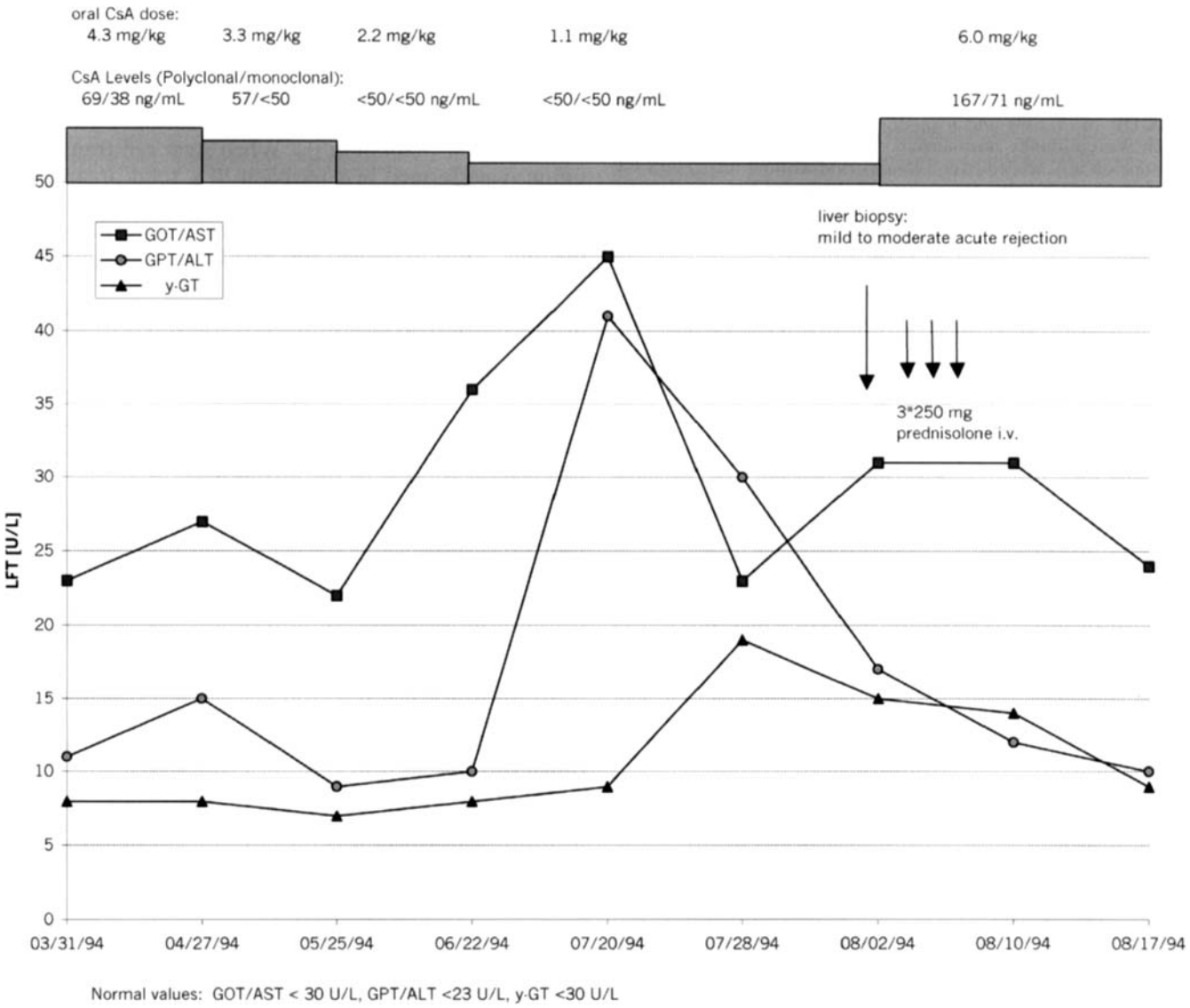

Fig. 1 Liver function tests while reducing immunosuppressive therapy. *GOT* Glutamic oxaloacetic transaminase, *AST* aspartate aminotransferase, *GPT* glutamic pyruvic transaminase, *ALT* alanine aminotransferase, *y-GT* γ-glutamyltranspeptidase

transplant is performed after solid organ transplant from different donors.

After BMT the immune system regularly becomes tolerant to the host, as evidenced by the fact that no immunosuppression is needed in most BMT patients after 6–12 months. In the presence of a foreign organ tolerance could include this organ. Indeed, in baboons tolerance has been induced by total lymphoid irradiation involving most of the recipient's BM [13]. Total lymphoid irradiation destroys mature lymphocytes. The immune system must develop in the presence of the foreign organ and becomes tolerant to the graft.

However, in our patient, although only very low immunosuppression was needed to maintain normal graft function, further reduction in CsA to polyclonal levels below 50 ng/ml led to rising LFT values. Liver biopsy showed rejection. This suggests that the developing immune system after BM transplantation does not develop tolerance towards a previously transplanted organ.

In conclusion, severe aplastic anemia after OLT and even rejection after BMT and OLT even in the presence of CMV does not necessarily carry a grim prognosis, and retransplantation can be carried out safely and with good success. A transplanted liver can tolerate even repeated courses of chemotherapy. Tolerance to the solid organ graft, however, does not seem to develop if BMT from a different donor is performed after OLT.

References

1. Abu-Elmagd K, Fung J, Todo S, Rao A, Reyes J, Demetris AJ, Mazariegos G, Fontes P, McMichael J, Furukawa H, Dodson F, Jain A, Pinna A, Marsh W, Marino I, Gayowski T, Doyle H, Madariaga J, Casavilla A, Corry R, Rakela J, Iwatsuki S, Starzl TE (1996) The current status of hepatic transplantation at the University of Pittsburgh. In: Terasaki PI, Cecka JM (eds) Clinical transplants 1995. UCLA Tissue Typing Laboratory, Los Angeles, pp 145–170
2. Cattral MS, Langnas AN, Markin RS, Antonson DL, Heffron TG, Fox IJ, Sorrell MF, Shaw BW Jr (1994) Aplastic anemia after liver transplantation for fulminant liver failure. Hepatology 20:813–818
3. Comenzo RL, Malachowski ME, Rohrer RJ, Freeman RB, Rabson A, Berkman EM (1992) Anomalous ABO phenotype in a child after an ABO-incompatible liver transplantation. N Engl J Med 326:867–870
4. Dey B, Sykes M, Spitzer TR (1998) Outcomes of recipients of both bone marrow and solid organ transplants. A review. Medicine (Baltimore) 77: 355–369
5. Fay JW, Wingard JR, Antin JH, Collins RH, Pineiro LA, Blazar BR, Saral R, Bierer BE, Przepiorka D, Fitzsimmons WE, Maher RM, Weisdorf DJ (1996) FK506 (tacrolimus) monotherapy for prevention of graft-versus-host disease after histocompatible sibling allogenic bone marrow transplantation. Blood 87:3514–3519
6. Garcia Morales R, Carreno M, Mathew J, Cirocco R, Zucker K, Ciancio G, Burke G, Roth D, Temple D, Fuller L, Esquenazi V, Eskind L, Kenyon NS, Ricordi C, Tzakis A, Miller J (1998) Continuing observations on the regulatory effects of donor-specific bone marrow cell infusions and chimerism in kidney transplant recipients. Transplantation 65:956–965
7. Haas RJ, Schmid I, Schon C, Soballa Stehr E, Stachel D (1999) Non-Hodgkin lymphoma after heart-lung transplantation: response to chemotherapy. Med Pediatr Oncol 32:229–230
8. Kanamaru A, Takemoto Y, Kakishita E, Dohy H, Kodera Y, Moriyama Y, Shibata A, Kasai M, Katoh S, Saitoh H (1995) FK506 treatment of graft-versus-host disease developing or exacerbating during prophylaxis and therapy with cyclosporin and/or other immunosuppressants. Japanese FK506 BMT Study Group. Bone Marrow Transplant 15:885–889
9. Kawahara K, Storb R, Sanders J, Petersen FB (1991) Successful allogeneic bone marrow transplantation in a 6.5-year-old male for severe aplastic anemia complicating orthotopic liver transplantation for fulminant non-A-non-B hepatitis. Blood 78:1140–1143
10. Langnas AN, Markin RS, Cattral MS, Naides SJ (1995) Parvovirus B19 as a possible causative agent of fulminant liver failure and associated aplastic anemia. Hepatology 22:1661–1665
11. Lee TH, Donegan E, Slichter S, Busch MP (1995) Transient increase in circulating donor leukocytes after allogeneic transfusions in immunocompetent recipients compatible with donor cell proliferation. Blood 85:1207–1214
12. Mazariegos GV, Reyes J, Marino IR, Demetris AJ, Flynn B, Irish W, McMichael J, Fung JJ, Starzl TE (1997) Weaning of immunosuppression in liver transplant recipients. Transplantation 63:243–249
13. Myburgh JA, Smit JA, Stark JH, Browde S (1984) Total lymphoid irradiation in kidney and liver transplantation in the baboon: prolonged graft survival and alterations in T cell subsets with low cumulative dose regimens. J Immunol 132:1019–1025
14. Nakamura T, Good RA, Yasumizu R, Inoue S, Oo MM, Hamashima Y, Ikehara S (1986) Successful liver allografts in mice by combination with allogeneic bone marrow transplantation. Proc Natl Acad Sci USA 83:4529–4532
15. Nash RA, Pineiro LA, Storb R, Deeg HJ, Fitzsimmons WE, Furlong T, Hansen JA, Gooley T, Maher RM, Martin P, McSweeney PA, Sullivan KM, Anasetti C, Fay JW (1996) FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. Blood 88: 3634–3641
16. Pardi DS, Romero Y, Mertz LE, Douglas DD (1998) Hepatitis-associated aplastic anemia and acute parvovirus B19 infection: a report of two cases and a review of the literature. Am J Gastroenterol 93:468–470
17. Ramos HC, Reyes J, Abu Elmagd K, Zeevi A, Reinsmoen N, Tzakis A, Demetris AJ, Fung JJ, Flynn B, McMichael J (1995) Weaning of immunosuppression in long-term liver transplant recipients. Transplantation 59:212–217
18. Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, Fay JW, Nademanee A, Antin JH, Christiansen NP, van der Jagt R, Herzig RH, Litzow MR, Wolff SN, Longo WL, Petersen FB, Karanes C, Avalos B, Storb R, Buell DN, Maher RM, Fitzsimmons WE, Wingard JR (1998) Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood 92:2303–2314
19. Ricordi C, Karatzas T, Nery J, Webb M, Selvaggi G, Fernandez L, Khan FA, Ruiz P, Schiff E, Olson L, Fernandez H, Bean J, Esquenazi V, Miller J, Tzakis AG (1997) High-dose donor bone marrow infusions to enhance allograft survival: the effect of timing. Transplantation 63:7–11
20. Selvaggi G, Ricordi C, Bottino R, Tzakis AG (1996) Liver transplantation without immunosuppression: future perspectives. Ital J Gastroenterol 28:246–249
21. Smit JA, Hill RR, Myburgh JA, Browde S (1980) Transplantation tolerance in primates after total lymphoid irradiation and allogeneic bone marrow injection. III. Lymphocyte responsiveness and suppressor cell activity. Transplantation 30:107–110

22. Trede NS, Warwick AB, Rosoff PM, Rohrer R, Bierer BE, Guinan E (1997) Tacrolimus (FK506) in allogeneic bone marrow transplantation for severe aplastic anemia following orthotopic liver transplantation. Bone Marrow Transplant 20:257–260

23. Tzakis AG, Arditi M, Whitington PF, Yanaga K, Esquivel C, Andrews WA, Makowka L, Malatak J, Freese DK, Stock PG, Ascher NL, Johnson FL, Broelsch CE, Starzl TE (1988) A plastic anemia complicating orthotopic liver transplantation for non-A, non-B hepatitis. N Engl J Med 319:393–396

24. Whitington PF, Rubin CM, Alonso EM, McKeithan TW, Anastasi J, Hart J, Thistlethwaite JR (1996) Complete lymphoid chimerism and chronic graft-versus-host disease in an infant recipient of a hepatic allograft from an HLA-homozygous parental living donor. Transplantation 62:1516–1519