Bone Marrow Transplantation, (1998) 22, 67–71

http://www.stockton-press.co.uk/bmt

Tetanus immunity in autologous bone marrow and blood stem cell transplant recipients

V Hammarstrom[1], K Pauksen[2], B Bjorkstrand[1], B Simonsson[3], G Oberg[3] and P Ljungman[1]

[1]*Department of Hematology, Huddinge University Hospital, Huddinge; and Departments of* [2]*Infectious Diseases and* [3]*Medicine, Uppsala University Hospital, Uppsala, Sweden*

Summary:

The aims of this study were to assess long-term immunity and reimmunization responses against tetanus toxoid in recipients of autologous stem cell grafts and to compare immune status in patients who underwent ABMT or autologous blood stem cell transplantation (APBSCT). Ninety patients were included in the study; 52 had received ABMT and 38 APBSCT. Thirty of 52 ABMT patients (58%) and 25 of 38 APBSCT patients (66%) had protective antibody levels against tetanus before transplantation (*P* = NS). The rate of seropositivity had decreased at 1 year after transplantation; 15 of 52 (29%) ABMT patients and 18 of 38 (47%) APBSCT patients (*P* = NS) were still positive after 1 year. There were no cases of spontaneous recovery in seronegative patients. Most patients were reimmunized with three doses of tetanus toxoid given at 12, 13, 14 and or 18 months after transplantation. All immunized patients had protective immunity against tetanus at 1 year after vaccination. These results suggest that humoral immunity is defective both after ABMT and after APBSCT and in both cases the loss of immunity seems to be similar. Reimmunization of patients who have undergone ABMT or APBSCT is necessary to obtain protective immunity against tetanus.

Keywords: tetanus immunity; marrow/stem cell transplantation

Tetanus is a disease completely preventable by vaccination.[1–5] The primary vaccination schedule with three doses of tetanus toxoid given at intervals of 4–6 weeks in the 2nd or 3rd month of life induces immunity lasting for up to 5 years. Each additional dose of tetanus toxoid given with at least 1 year interval increases antibody levels and duration of immunity.[6–10] In most developed countries a 4 or 5 dose vaccination schedule is used which induces high and durable immunity. However, tetanus immunity is not life-long and with ageing a decrease in antibody levels is seen.[11–14] Our previous studies of tetanus immunity showed that long-term survivors after allogeneic BMT lose protective levels of tetanus antibodies and thus require reimmunization with a schedule similar to that used in children.[15,16] Similarly, patients treated with cytotoxic chemotherapy for hematological malignancies are frequently unprotected against tetanus.[17] However, patients undergoing ABMT do not lose pretransplant immunity against pneumococci[18] and a majority of the patients remain seropositive (79–84%) to rubella, measles, mumps and polio at 1 year after ABMT.[19,20] APBSCT is rapidly replacing ABMT for patients with solid tumors and hematological malignancies. One advantage of APBSCT over ABMT is a more rapid hematopoietic recovery and it might be that the defect in humoral immunity is less profound after APBSCT than after ABMT.[21,22]

The aims of this study were to assess long-term immunity to tetanus toxoid among patients who have undergone ABMT and APBSCT and to assess the response to reimmunization with tetanus toxoid.

Materials and methods

Patients

We studied antibody levels against tetanus in 52 ABMT and 38 APBSCT patients. All patients had a disease-free survival of at least 24 months after the transplantion. The age distribution and diagnoses are presented in Table 1. Nine APBSCT patients were transplanted with $CD34^+$-purged grafts. The CD34 selection was carried out with the Ceprate column (Cell-Pro, Bothell, WA, USA). Two of these patients had multiple myeloma (MM), six had breast cancer (BC) and one had CLL. Three patients with MM and one with BC underwent double APBSCT procedures.

Table 1 Number of ABMT and ABSCT patients with different diagnoses

Diagnosis	*ABMT*	*ABSCT*
Acute myeloid leukemia	14	0
Acute lymphoblastic leukemia	15	0
Non-Hodgkin's lymphoma	9	6
Hodgkin's disease	9	0
Multiple myeloma	5	16
Chronic lymphatic leukemia	0	4
Breast cancer	0	12
Age	31 (3–61)	44 (17–62)

Correspondence: Dr V Hammarstrom, Department of Hematology, Huddinge University Hospital, 141 86 Huddinge, Sweden
Received 12 November 1997; accepted 14 February 1998

ABMT and APBSCT procedure

In all patients, the marrow was harvested at the same stage of disease as it was reinfused. The ABMT and APBSCT procedures have been described previously.[23–25] Briefly, patients with AML were conditioned with cyclophosphamide (Cy) 120 mg/kg and either single fraction of total body irradiation (TBI) (7.5 or 10 Gy) or busulphan 16 mg/kg. Patients with ALL were conditioned with Cy 80 mg/kg plus a combination of vincristine 1.5 mg/m^2, daunorubicin 30 mg/m^2, tenoposide 200 mg/m^2, cytosine arabinoside (Ara-C) 2500 mg/m^2, prednisone 200 mg/m^2 and TBI 7.5 Gy. Most patients with NHL were conditioned with either the BEAC or BEAM protocol. Most patients with HD were conditioned with carmustine 300 mg/m^2, etoposide 300 mg/m^2, methyl GAG 600 mg/m^2 and Cy 100 mg/kg. A few patients with NHL and HD had additional TBI. Patients with multiple myeloma were conditioned with Cy 400 mg/m^2, methylprednisolone 8 g, melphalan 140 mg/m^2 and TBI 7.5 Gy, or melphalan 140 mg/m^2 and TBI 10 Gy, or melphalan 200 mg/m^2 given as a single agent. Patients with breast cancer were conditioned with the Stamp V protocol (Cy 6000 mg/m^2, thioteopa 500 mg/m^2 and carboplatin 800 mg/m^2 (CTCb)) given over 4 consecutive days. Patients with CLL were conditioned with Cy 60 mg/kg and TBI. Three patients with MM and one with BC underwent double APBSCT procedures. The first preparative regimen was high dose melphalan followed by cyclophosphamide + TBI in the myeloma patients and CTCb in the breast cancer patients. The blood stem cells were harvested with a Fenwal CS-3000 Plus (Baxter, Chicago, IL, USA) usually after chemotherapy and growth factor stimulation.[26] Most patients were stimulated with G-CSF before harvest, two patients with NHL and one patient with CLL were not stimulated with any growth factor. CD34$^+$-purged graft was used in two patients with MM, six patients with BC and one patient with CLL. The minimal number of CD34$^+$ cells required for transplantation was 2×10^6/kg.

Serology

An enzyme linked immunosorbent assay (ELISA) was used to determine antibody levels against tetanus toxoid. The ELISA has been described previously.[27–29] Briefly, after incubation of serum samples (diluted 1:100–1:10 000) overnight on antigen-coated plates, a monoclonal mouse anti-IgG-antibody (HP 06; Seward Laboratories, London, UK) was added in a previously determined optimal concentration. After 4 h of incubation at room temperature the plates were washed and rabbit anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark) was added. After another 4 h of incubation the plates were washed and alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical, St Louis, MO, USA) was added, after which the plates were incubated overnight. After additional washes, disodium *p*-nitrophenyl phosphate (Sigma Chemical) was added and the plates incubated for 10–20 min. Absorbance was measured at 405 nm.

Reference of anti-tetanus antibodies

A standard curve was constructed using tetanus IgG standard preparation containing 190 IU/ml of tetanus toxoid antibodies, calibrated against the International standard serum, purchased from the Swedish Institute for Infectious Disease Control, Stockholm. For a minimal protective level of tetanus antibodies the WHO recommendation 0.01 IU/ml was used.[4]

Samples and immunization

Serum samples from before ABMT or APBSCT and from 1 and 2 years after transplantation were analyzed.

ABMT and APBSCT patients were reimmunized with three doses of tetanus toxoid (National Bacteriological Laboratory-Vaccine, Stockholm, Sweden) given at 12, 13 and 14 or 18 months after transplantation. Five ABMT patients were reimmunized later, at 18, 19 and 20 months post transplant, after previous determination of tetanus antibodies.

Statistics

The Mann–Whitney test was used to compare ages in ABMT and APBSCT patients and in patients with BC and hematologic malignancies.

The proportions of ABMT and APBSCT patients immune to tetanus before and after transplantation and rates of seropositivity in patients with different diagnoses were compared in 2×2 contingency tables using Fisher's exact test.

The multivariate logistic regression was used to compare antibody levels and age of the patients as a continuous variable, type of disease, type of graft and CD34 stem cell separation.

Results

Tetanus antibodies in ABMT patients

Thirty of 52 ABMT patients examined (58%) were seropositive against tetanus before transplantation. At 12 months after transplantation, 15 patients remained positive, while 15 patients had lost their pretransplant immunity. No patient who was seronegative before transplantation seroconverted during the first year after transplantation. There was no difference in seropositivity at 12 months in patients who were conditioned with or without TBI (data not shown). The numbers of seropositive patients before transplantation and at 1 year after ABMT analyzed according to diagnoses are presented in Table 2. There was no differ-

Table 2 Number of seropositive patients/total ABMT patients before and 1 year after transplantation

	Before transplantation	*After transplantation*	*P value*
Acute lymphoblastic leukemia	8/15	4/15	0.14
Acute myeloid leukemia	10/14	6/14	0.13
Multiple myeloma	3/5	1/5	0.26
Non-Hodgkin's lymphoma	4/9	2/9	0.31
Hodgkin's disease	5/9	2/9	0.17

ence in proportions of patients being immune before ABMT or in the rates of loss of immunity between patients with different underlying diagnoses.

Tetanus antibodies in APBSCT patients

Twenty-five of 38 (66%) APBSCT patients were seropositive against tetanus before transplantation. At 12 months after transplantation, 18 (47%) patients remained seropositive and seven (19%) patients had lost pretransplant tetanus immunity.

Ten of 12 breast cancer patients were seropositive before transplantation (83%). Two patients lost pretransplant immunity. Thus, at 1 year eight patients (67%) remained seropositive against tetanus.

Fifteen of 26 (57%) patients with hematological malignancies were seropositive before transplantation and 10 (38%) remained immune at 1 year after transplantation. There was no difference in the proportions of breast cancer patients and patients with hematological malignancies who were immune before transplantation (P = NS). Furthermore, there was no difference between the different patient categories in capacity to retain immunity at 1 year after transplant (P = NS; Table 3).

Comparison of pre- and post-transplant tetanus immunity in ABMT patients and APBSCT recipients

The proportions of immune patients before transplantation in the two patient populations were similar (58 and 66% in ABMT and APBSCT, respectively). There was a tendency for patients who had received APBSCT to retain immunity better than patients who had received an autologous marrow graft ($P = 0.08$). However, the antibody levels in both groups decreased in a similar fashion during the first year after transplantation (Figures 1 and 2). In a multivariate logistic regression model none of the analyzed factors (diagnosis ($P = 0.18$), age ($P = 0.25$), type of graft ($P = 0.73$) or CD34-selected stem cells ($P = 0.72$)) influenced likelihood for loss of immunity to tetanus.

Immunization responses

All reimmunized patients responded with a significant increase in the level of specific anti-tetanus IgG and were immune when studied at 24 months after transplantation (Figures 1 and 2).

Table 3 Number of seropositive/total ABSCT patients before and 1 year after transplantation

	Before transplantation	*After transplantation*	*P value*
Multiple myeloma	9/16	6/16	0.23
Chronic lymphatic leukemia	2/4	1/4	0.5
Hodgkin's disease	4/6	3/6	0.5
Breast cancer	10/12	8/12	0.32

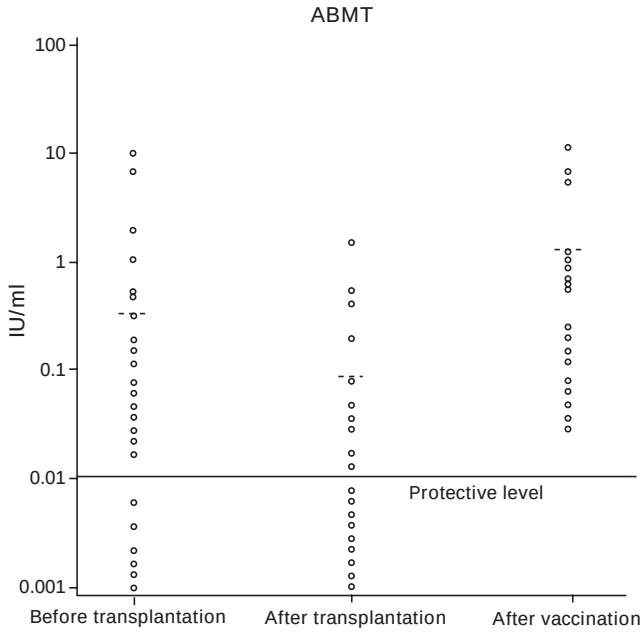

Figure 1 Tetanus immunity in ABMT patients before transplantation, 1 year after transplantation and 1 year after reimmunization with tetanus toxoid.

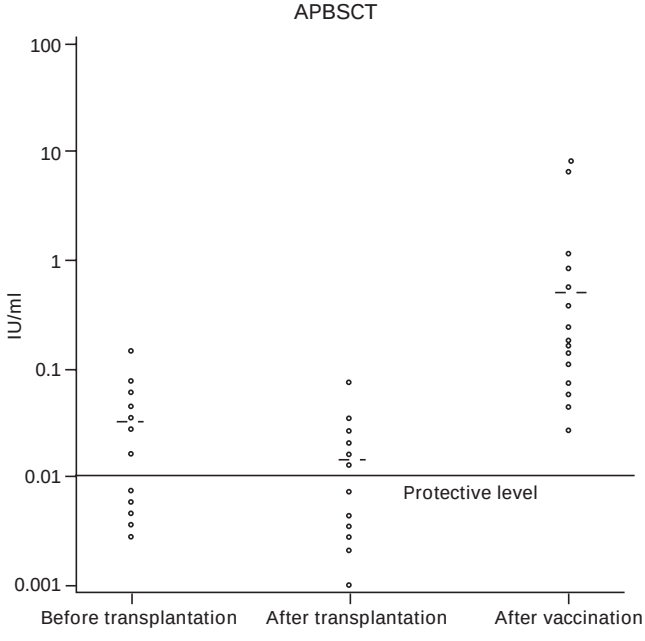

Figure 2 Tetanus immunity in APBSCT patients before and 1 year after transplantation and 1 year after reimmunization with tetanus toxoid.

Discussion

ABMT is generally thought to be accompanied by less profound immunodeficiency than allogeneic BMT. This might be explained by the absence of acute and chronic graft-versus-host disease (GVHD) and absence of immunosuppression as prevention and treatment of GVHD. Studies of long-term survivors after allogeneic BMT have shown that approximately 50% of BMT recipients lost specific

immunity to infectious agents such as measles, tetanus, pneumococci and diphtheria and about one-third of patients lost immunity against polio during the first 12 months after BMT.[17,30–33]

In our study 42% and 33% of ABMT and APBSCT recipients, respectively, had no tetanus immunity prior to transplantation. It was found previously that increasing age, lymphoid malignancy, and advanced stage of disease were independent risk factors for loss of tetanus immunity in patients with hematologic malignancies.[17,34,35] In the current study tetanus immunity decreased to 50% in ABMT patients. Thus, the loss of tetanus immunity in ABMT patients was almost as frequent during the first year after transplant as in BMT patients.[15]

Previous studies of specific immunity after ABMT showed that risk for losing immunity varied between different antigens tested: patients usually retained pretransplant levels of pneumococcal antibodies,[18,36] but frequently lost immunity against polio.[19] Immunity against pneumococci is obtained through natural immunization, whereas immunity against polio and tetanus is induced by active immunization. It was shown previously[3,4] that natural immunization induces higher and more durable immunity than vaccination for measles, mumps and rubella. Whether APBSCT patients retain specific immunity differently from ABMT patients has been mostly unknown. Rapid hematological recovery could suggest that immunological recovery would also be earlier.

In our study 28% of APBSCT patients who were seropositive before transplantation lost their pretransplant immunity during the first year post transplant. There was no significant difference in the capacity to retain tetanus immunity between ABMT and APBSCT. The APBSCT patients were significantly older ($P = 0.0001$) but in a multivariate model neither age nor the other factors examined had any significant impact on loss of immunity. However, taking into consideration that APBSCT patients were older and there was a tendency to retain immunity better in this group, it is possible that due to the small size of our study a significant difference might have been missed.

In conclusion, tetanus immunity is deficient after both ABMT and APBSCT, and the use of APBSCT does not seem to substantially improve long-term immunity to tetanus since 71% of ABMT patients and 53% of APBSCT patients were unprotected against tetanus 1 year after transplantation. Reimmunization with tetanus toxoid should be considered in both ABMT and APBSCT patients, since there is no evidence supporting spontaneous recovery of tetanus immunity in healthy individuals,[4] in HIV-infected patients[37] or BMT patients.[15]

All reimmunized patients in our study responded with a significant increase of tetanus antibodies and were seropositive on long-term follow-up. Immunological memory and the ability to respond quickly to booster doses of tetanus toxoid[4] may be as important as the level of circulating antibodies in determining the outcome of infection with tetanus. It was previously shown that a reimmunization schedule with three doses of tetanus toxoid is necessary to induce protective and durable immunity[15] in BMT patients. The mechanisms for loss of immunity in ABMT patients seems to be similar to BMT and therefore a three dose schedule should also be used in autologous transplant patients. Acceleration of hematologic reconstitution after APBSCT predicts that reimmunization could be provided earlier and the period of deficiency of specific immunity could be shorter.

Acknowledgements

This work was supported by grants from the Swedish Children's Cancer Foundation and Swedish Cancer Society.

References

1 Veronesi R. Epidemiology of tetanus. In: L Eckmann (ed). *Principles of Tetanus* Hans Huber: Bern, 1967, pp 43–48.
2 Bytchenko B. Tetanus as a world problem. In: Eckman L (ed). *Principles of Tetanus*. Hans Huber: Bern, 1967, pp 21–41.
3 Eckman L. Active and passive tetanus immunization. *New Engl J Med* 1964; **271**: 1087–1091.
4 Galazka AM. The immunological basis for immunization. Module 3: tetanus. In: *Expanded Programme on Immunization*. World Health Organization: Geneva, 1993.
5 Eckman L. *Tetanus: Prophylaxis and Therapy*. Grune & Straton: New York, 1963, pp 50–53.
6 Christenson B, Bottiger M. Epidemiology of tetanus in Sweden. *Scand J Infect Dis* 1987; **19**: 1429–1435.
7 Anderson EL, Belshe RB, Bartram J. Differencies in reactogenicity and antigenicity of acellular and standard pertussis vaccines combined with diphtheria and tetanus in infants. *J Infect Dis* 1988; **157**: 731–737.
8 Barkin RM, Samuelson JS, Gotlin LP. DTP reactions and serologic response with reduced dose schedule. *J Pediatr* 1984; **105**: 189–194.
9 Pichichero ME, Barkin RM, Samuelson S. Pediatric diphtheria and tetanus toxoids-adsorbed vaccine: immune response to the booster following the diphtheria and tetanus toxoid vaccine primary series. *Ped Inf Dis J* 1986; **5**: 428–430.
10 Barkin RM, Samuelson JS, Gotlin LP. Diphtheria and tetanus toxoids and pertussis vaccine adsorbed (DTP): response to varying immunizing dosage and schedule. *Dev Biol Stand* 1985; **61**: 297–307.
11 Simonsen O, Badsberg JH, Kjelsdsen K *et al*. The fall-off in serum concentration of tetanus antitoxin after primary and booster vaccination. *Acta Pathol Microbiol Immunol Scand* 1986; **94**: 77–82.
12 Kjeldsen K, Simonsen O, Heron I. Immunity against diphtheria and tetanus in the age group 30–70 years. *Scand J Inf Dis* 1988; **20**: 177–185.
13 Simonsen O, Bentzon MW, Kjeldsen K *et al*. Evaluation of vaccination requirements to secure continuous antitoxin immunity to tetanus. *Vaccine* 1987; **5**: 115–122.
14 Galazka A, Sporzynska Z. Immunity to tetanus and diphtheria in various age groups of the Polish population. *Arch Immunol Ther Exp* 1979; **27**: 715–726.
15 Ljungman P, Wiklund-Hammarsten M, Duraj V *et al*. Response to tetanus toxoid immunization after allogeneic bone marrow transplantation. *J Infect Dis* 1990; **162**: 496–500.
16 Zander AR, Reuben JM, Johnstone D *et al*. Immune recovery following allogeneic bone marrow transplantation. *Transplantation* 1985; **40**: 177–183.
17 Hammarstrom V, Pauksen K, Bjorkstrand B *et al*. Tetanus immunity in patients with hematological malignancies and in autologous stem cell transplant (ASCT) recipients. *Blood* 1995; **86** (Suppl. 1): 105a (Abstr. 408).

18 Hammarstrom V, Pauksen K, Azinge J *et al.* Pneumococcal immunity and response to immunization with pneumococcal vaccine in bone marrow transplant recipients; the influence of graft versus host reaction. *Supp Care Cancer* 1993; **1**: 195–199.
19 Pauksen K, Hammarstrom V, Ljungman P *et al*. Immunity to poliovirus and immunization with inactivated poliovirus vaccine after autologous bone marrow transplantation. *Clin Infect Dis* 1994; **18**: 547–552.
20 Pauksen K, Duraj V, Ljungman P *et al*. Immunity to and immunization against measles, rubella and mumps in patients after autologous bone marrow transplantation. *Bone Marrow Transplant* 1992; **9**: 427–432.
21 Roberts MM, To LB, Gillis D *et al*. Immune reconstitution following peripheral blood stem cell transplantation, autologous bone marrow transplantation and allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1994; **13**: 377–381.
22 Rosillo MC, Ortuno F, Moraleda JM *et al.* Immune recovery after autologous or rhG-CSF primed PBSC transplantation. *Eur J Haematol* 1996; **14**: 219–223.
23 Simonsson B, Burnett AK, Prentice HG *et al.* Autologous bone marrow transplantation with monoclonal antibody purged marrow for high risk acute lymphoblastic leukemia. *Leukemia* 1989; **3**: 631–636.
24 Tutschka PJ, Copelan EA, Klein JP. Bone marrow transplantation for leukemia following a new busulphan and cyclophosphamide regimen. *Blood* 1987; **8**: 1382–1388.
25 Lonnerholm G, Carlsson K, Bratteby LE *et al*. Renal function after autologous bone marrow transplantation. *Bone Marrow Transplant* 1991; **8**: 129–134.
26 Gianni AM, Siena S, Bregni M *et al.* Granulocyte–macrophage colony-stimulating factor to harvest circulating haematopoetic stem cells for autotransplantation. *Lancet* 1989; **66**: 416–422.
27 Person MAA, Hammarstrom L, Smith CIE. Enzyme linked immunosorbent assay for subclass distribution IgG and IgA antigen-specific antibodies. *J Immunol Meth* 1985; **78**: 109–115.
28 Simonsen O, Bentzon MW, Heron I. ELISA for the routine determination of antitoxic immunity to tetanus. *J Biol Stand* 1986; **14**: 231–239.
29 Sedgwick AK, Balow M, Sparks K *et al.* Rapid quantitative microenzyme-linked immunosorbent assay for tetanus antibodies. *J Clin Microbiol* 1983; **18**: 104–109.
30 Saxon A, Mitsuyasu R, Stevens R *et al*. Designed transfer of specific immune responses with bone marrow transplantation. *J Clin Invest* 1986; **78**: 959–967.
31 Ljungman P, Cordonnier C, De Bock R *et al.* Immunizations after bone marrow transplantation: results of a European survey and recommendations from the infectious diseases working party of the European Group for Blood an Marrow Transplantation. *Bone Marrow Transplant* 1995; **15**: 455–460.
32 Somani J, Larson RA. Reimmunization after allogeneic bone marrow transplantation. *Am J Med* 1995; **98**: 389–398.
33 Quinti I, Velardi A, Le Moli S *et al*. Antibacterial polysaccharide antibody deficiency after allogeneic bone marrow transplantation. *J Clin Immunol* 1990; **10**: 160–166.
34 Kishimoto S, Tomino S, Mitsuya H *et al.* Age-related decline *in vitro* and *in vivo* synthesis of anti-tetanus antibodies in humans. *J Immunol* 1980; **125**: 2347–2352.
35 Mackall CL, Fleisher TA, Brown MR *et al.* Age, thymopoiesis and $CD4^+$ T-lymphocyte regeneration after intensive chemotherapy. *New Eng J Med* 1995; **332**: 143–149.
36 Lum LG, Burns E, Janson MM *et al*. IgG anti-tetanus toxoid antibody synthesis by human bone marrow. Two distinct populations of marrow and peripheral blood B cells. *J Clin Immunol* 1990; **10**: 255–264.
37 Opravil M, Matter L, Blaser J *et al*. Poor antibody response after tetanus and pneumococcal vaccination in immunocompromised, HIV-infected patients. *Clin Exp Immunol* 1991; **84**: 185–189.