

Anti-Tetanus Toxoid Antibody Production and Protection against Lethal Doses of Tetanus Toxin in hu-PBL-SCID Mice

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Key Words

hu-PBL-SCID • Tetanus • Protection • Vaccine • Evaluation

Abstract

Background: In this study, severe combined immunodeficiency (SCID) mice, which permit the survival of lymphoid cells of human origin, were used to study the human anti-tetanus immune response. **Methods:** Human peripheral blood lymphocytes (hu-PBL) obtained from 88 healthy donors (aged from 18 to 62) were transplanted into SCID mice, and anti-tetanus toxoid (Ttd) antibody production and protection against lethal doses of tetanus toxin (Ttx) were investigated in the hu-PBL-SCID mice. **Results:** The transfer of human PBL evoked significant human anti-Ttd IgG antibody production for 37.5% of the donors. After in vivo immunization, the percentage of donors with PBL exhibiting positive anti-Ttd IgG production in the mice increased to 54.5%. Mean anti-Ttd IgG levels in the sera were also significantly elevated in response to immunization. The mean IgG titer for the mice injected with PBL from donors under the age of 40 was significantly higher than that of the mice injected with PBL from donors aged 40 or older. Four

weeks after the cell transfer, the mice were challenged with Ttx. The induction of protection against Ttx challenge was observed mostly in mice with PBL transferred from donors under the age of 40. In vivo immunization in SCID mice with Ttd increased the number of cases of resistance to Ttx. **Conclusions:** These results suggest that hu-PBL-SCID mice might serve as a tool for predicting the protective ability against pathogens in PBL donors and also for evaluating vaccine efficacy.

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Introduction

Vaccines are the most efficient tool for controlling or eliminating severe infectious diseases. Reductions in mortality attributable to diphtheria, whooping cough, tetanus, small pox, measles and polio have been achieved over recent decades using vaccines. Today, vaccine efficacy and safety are in many cases evaluated using experimental animals. However, the results obtained do not always correlate with the reaction caused by a vaccine in humans. Therefore, an improved protocol of evaluation that represents human reactivities is required. Bosma et

al. [1] established a model of severe combined immunodeficiency (SCID) mice, namely C.B-17 *scid/scid* mice. Since they permit the survival of lymphoid cells of human origin, the use of SCID mice has made it possible to reconstitute a functional human immune system in mice injected with human peripheral blood leukocytes (hu-PBL) [2]. This hu-PBL-SCID system has been demonstrated to be useful for the analysis of HIV-1 infection in humans [3], for making human antibodies [4–6], and for the analysis of human T and B cell functions [7]. Not only secondary responses, but also primary humoral responses have been demonstrated to be induced in hu-PBL-SCID mice [8]. Upon vaccination *in vivo*, an increased resistance to infection has been observed [9].

In this study, SCID mice were transplanted with PBL from 88 healthy human donors from 18 to 62 years of age and were immunized either with tetanus toxoid (Ttd) or nothing at all. Four weeks after the cell transfer, mice were challenged with a lethal dose of tetanus toxin (Ttx) and protective ability was monitored. The relationship between anti-Ttd IgG antibody production in SCID mice and donor ages, and the correlation between specific IgG antibody production and protective ability against lethal doses of tetanus toxin were investigated. The aim of this study was to evaluate the potential of the hu-PBL-SCID model for predicting the protective ability against pathogens in PBL donors and for evaluating vaccine efficacy.

Materials and Methods

Mice

Female SCID (C.B-17/1crCrj-*scid/scid*) and BALB/c mice, 6 weeks of age, were purchased from the Charles River Co., Kanagawa, Japan. Mice were maintained in sterile cages under specific pathogen-free conditions at the Division of Experimental Animal Research (National Institute of Infectious Diseases, Tokyo, Japan),

Cell Separation and Repopulation of SCID Mice

Peripheral blood was obtained from blood donated by 88 healthy donors of both sexes ranging in age from 18 to 62 years. PBL were prepared by density centrifugation on HistopaqueTM-1077 (Sigma Diagnostics Inc., St. Louis, Mo., USA) of buffy coats from donors. The PBL were resuspended in sterile phosphate-buffered saline (PBS; pH 7.2) and injected (2×10^7 cells in 500 μ l of PBS) intraperitoneally into SCID mice.

Tetanus Toxin and Tetanus Toxoid

For the preparation of Ttx, culture supernatant of *Clostridium tetani* (Harvard A-47 strain) was concentrated by ultrafiltration, salted out twice with ammonium sulfate, and purified by ion exchange chromatography. Ttd was prepared by detoxifying the purified Ttx using formaldehyde, followed by dialysis against PBS.

In vivo Immunization

hu-PBL-SCID mice were immunized subcutaneously with 10 μ g of Ttd in 3 mg of aluminum hydroxide (Ttd-alum) in 200 μ l of PBS immediately after cell transfer.

Measurement of Serum Antibodies

Three weeks after cell transfer, mice were bled from the tail vein. Human IgG antibodies against Ttd in the mice sera were measured using ELISA. Briefly, Ttd-specific ELISAs were performed by coating 96-well MaxisorpTM immuno plates (Cat. No. 442404, Nalgenunc Int. Co., Tokyo, Japan) with 5 μ g of Ttd/ml in 0.1 M carbonate buffer (pH 9.0). The remaining binding sites were blocked with 5% human serum albumin (Bayer Co., Kankakee, Ill., USA) in PBS. Human IgG antibodies bound to the plate were detected using peroxidase-goat anti-human IgG (Zymed Lab. Inc., San Francisco, Calif., USA).

Ttx Challenge

Four weeks after cell transfer, SCID mice and age-matched naive BALB/c mice were challenged subcutaneously with $10 \times \text{LD}_{50}$ of Ttx, i.e. 100 ng of purified Ttx in 500 μ l of PBS. After the challenge, the age-matched nonimmunized control mice died within 24 h. A group of mice was judged as protected against Ttx when all five mice in the group were alive for more than 1 week without any apparent symptoms.

Statistical Analysis

Student's *t* test was employed for the statistical analysis.

Results

Relationship between Anti-Ttd IgG Production in hu-PBL-SCID Mice and Age of PBL Donor

PBL from a donor were injected into 10 SCID mice (2×10^7 cells per mouse). Five mice were subsequently immunized subcutaneously with Ttd-alum, and the other 5 mice were left untreated. The levels of anti-Ttd IgG antibody titers in SCID mice reached a plateau 3 weeks after cell transfer. Figure 1a shows the relationship between anti-Ttd IgG titers and the ages of the 88 PBL donors. SCID mice injected with 48 PBL donors produced anti-Ttd IgG with or without *in vivo* immunization, while mice injected with 15 PBL donors produced antibodies only with immunization. The rest of the mice (25 PBL donors) did not produce antibodies, regardless of immunization. Anti-Ttd IgG production seems to be more evident in mice injected with PBL from younger donors than in mice injected with PBL from older donors. For instance, from 55 PBL donors under the age of 40, 35 donors' PBL (63.6%) induced anti-Ttd IgG in SCID mice without immunization, and 48 donors' PBL (87.3%) did so after immunization. On the other hand, from 33 PBL donors aged 40 or older, 13 donors' PBL (39.3%) produced antibodies in mice without immunization, and 15

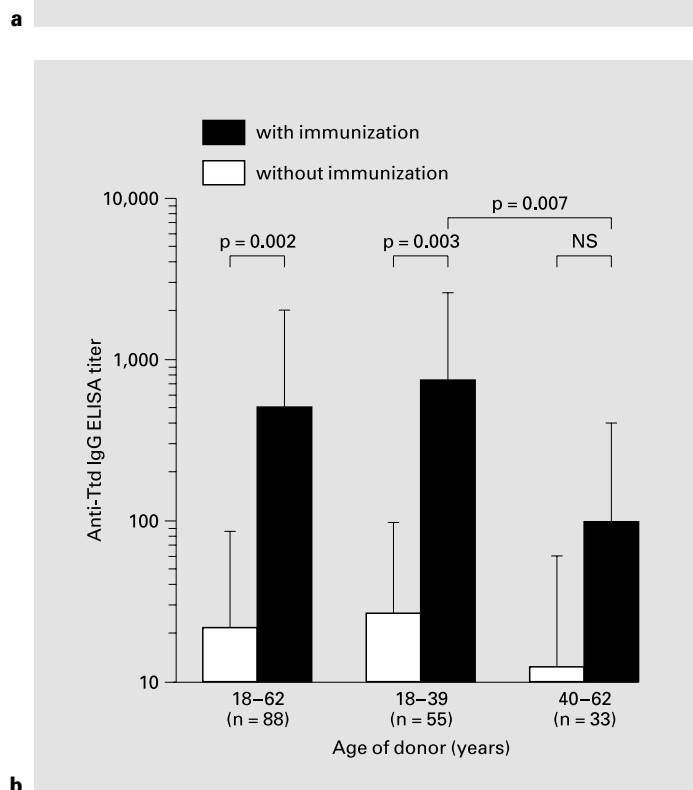
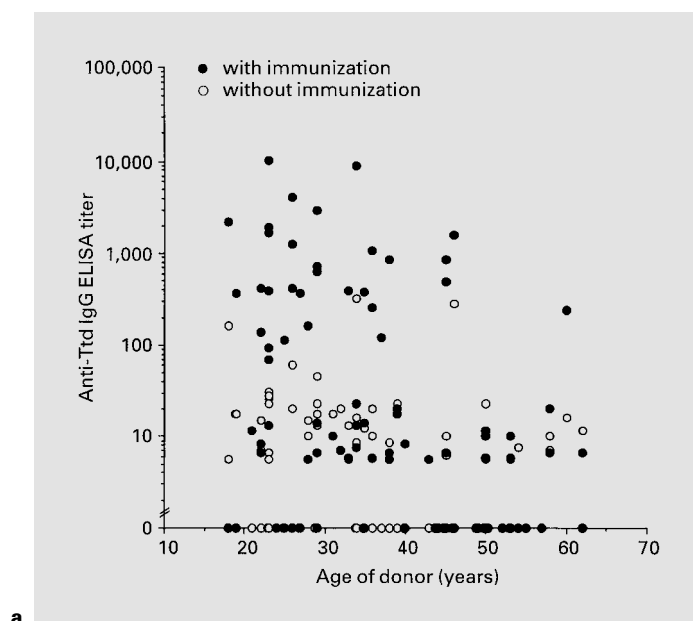


Fig. 1. Serum anti-Ttd titer in hu-PBL-SCID mice. Three weeks after cell transfer, mice were bled from the tail vein, and serum anti-Ttd IgG titer was measured. **a** Mean antibody titer of 5 mice per group. A value of 0 for anti-Ttd IgG ELISA titer indicates undetectable levels. **b** Mean antibody titer and SE of groups of mice divided according to donor age. NS = Not significant.

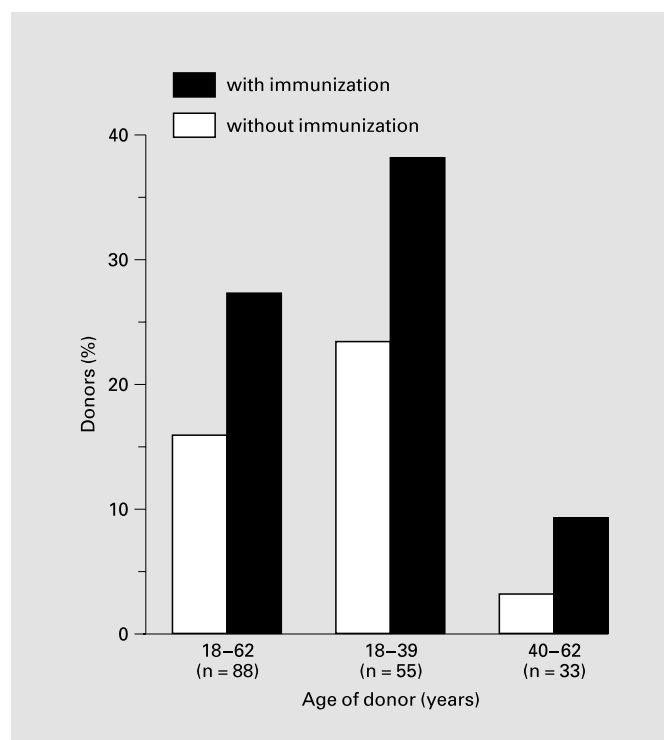


Fig. 2. Percentage of donors that induced protection against Ttx challenge in hu-PBL-SCID mice.

donors' PBL (45.5%) did so after immunization. The effect of Ttd-alum immunization on the anti-Ttd IgG titers was also noticeable in mice injected with PBL from younger donors. In fact, the mean IgG titer of the mice injected with PBL from donors under the age of 40 was significantly higher ($p < 0.01$) than that of the mice injected with PBL from donors aged 40 or older (fig. 1b). Furthermore, the elevation of anti-Ttd IgG titers induced by in vivo immunization was significant in the mice as a whole and in the mice injected with PBL from donors under the age of 40, but not in the mice injected with PBL from donors aged 40 or older (fig. 1b).

Protection against Challenge with a Lethal Dose of Ttx in hu-PBL-SCID Mice

Four weeks after cell transfer, the hu-PBL-SCID mice were challenged subcutaneously with $10 \times LD_{50}$ of Ttx, and symptoms appearing in the mice were monitored thereafter. As shown in figure 2, the induction of protection against Ttx challenge was observed mostly in the group of mice with PBL transferred from donors under 40. In vivo immunization of SCID mice with Ttd increased the number of cases of protection; in mice

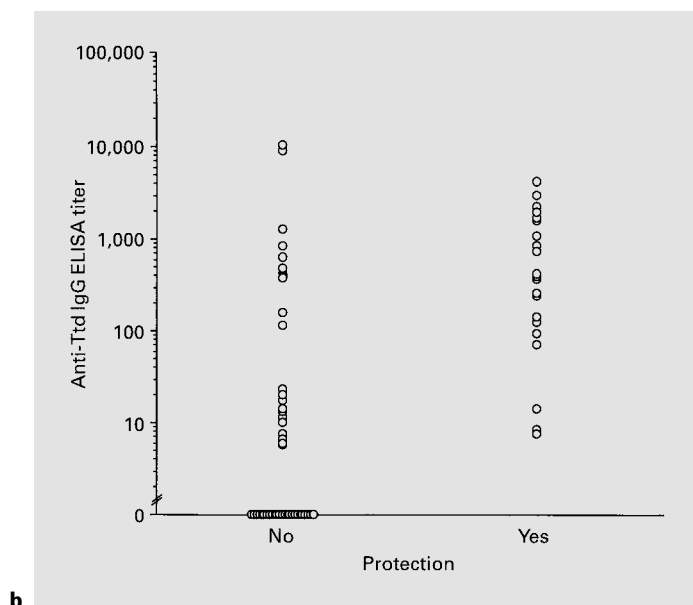
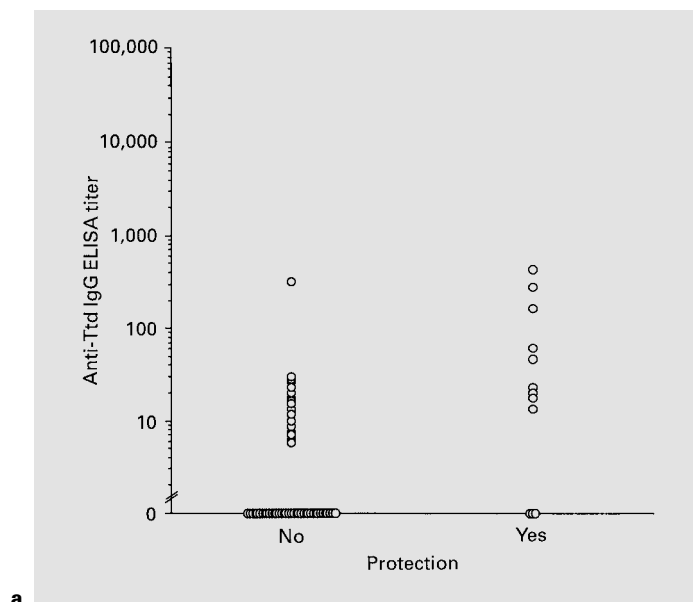


Fig. 3. Serum anti-Ttd IgG titers in hu-PBL-SCID mice that did or did not protect against Ttx challenge. **a** Without in vivo immunization. **b** With in vivo immunization. A value of 0 for anti-Ttd IgG ELISA titer indicates undetectable levels. Data represent the mean antibody titer of 5 mice per group.

injected with PBL from donors under 40, the number of cases in which protection was induced was 13 (23.6%) without immunization, and 21 (38.2%) with immunization, whereas in mice with PBL transferred from donors aged 40 or older, the number was 1 (3.0%) without immunization and 3 (9.1%) with immunization.

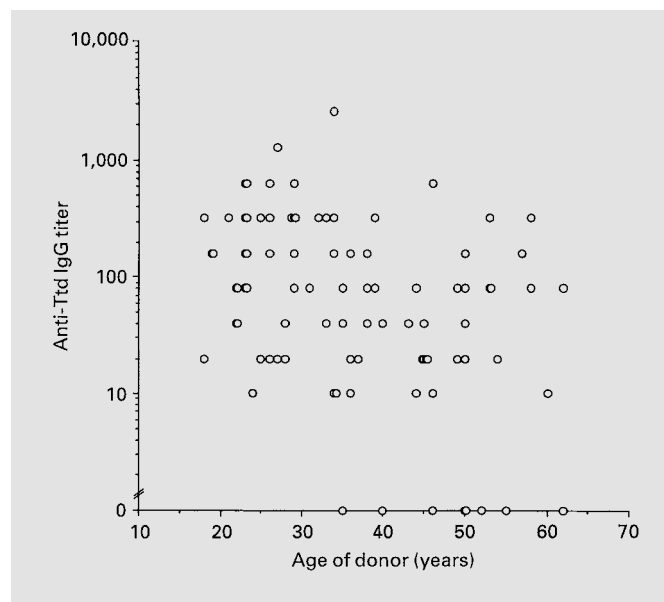


Fig. 4. Relationship between serum anti-Ttd IgG titer in PBL donor and donor age. A value of 0 for anti-Ttd IgG titer indicates undetectable levels.

Correlation between Anti-Ttd IgG Production and Protection against Ttx in hu-PBL-SCID Mice

Figure 3 shows the anti-Ttd IgG antibody titers in SCID mice protected against Ttx challenge and in those which were not. The median values of anti-Ttd IgG titers in the groups of protected and unprotected mice, respectively, were 19 (range 1–422) and 3 (range 1–320) without immunization (fig. 3a), and 381 (range 8–4,159) and 6 (range 1–10,240) with immunization (fig. 3b). Interestingly, in the group of mice that received no immunization, 3 cases were found in which anti-Ttd IgG was undetectable, although complete protection against Ttx was induced (fig. 3a). A discrepancy between the serum anti-Ttd IgG titer and protection against Ttx was also observed in mice receiving immunization; there were 2 cases in which high titers of serum anti-Ttd IgG were detected, but there was a lack of protection against Ttx (fig. 3b).

Relationship between Serum Anti-Ttd IgG Titer in PBL Donors and Age of Donors

Figure 4 shows the relationship between anti-Ttd IgG titer in donor serum and donor age. Anti-Ttd IgG titer differed significantly ($p < 0.003$) between the younger (under 40) and older (40 or older) groups. In 85% of the

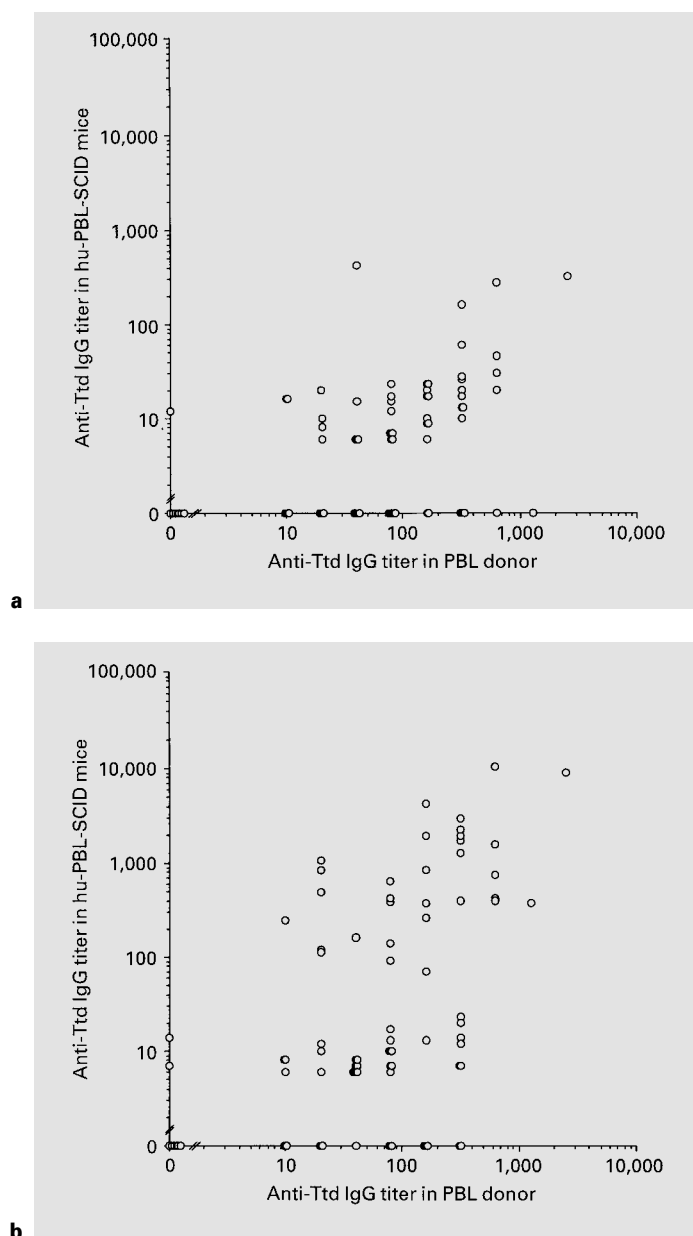


Fig. 5. Correlation between serum anti-Ttd IgG titers in PBL donors and hu-PBL-SCID mice. **a** Without in vivo immunization. **b** With in vivo immunization. Data for serum anti-Ttd IgG in hu-PBL-SCID mice represent the mean antibody titer of 5 mice per group. A value of 0 for anti-Ttd IgG titer indicates undetectable levels.

donors in the older group, anti-Ttd IgG titers were less than 100, while in the younger group, titers were over 100 in 51% of the donors. The median values of anti-Ttd IgG titers were 160 (range 5–2,560) in the younger group and 20 (range 5–640) in the older group.

Correlation between Serum Anti-Ttd IgG in PBL Donors and hu-PBL-SCID Mice

Figure 5 shows the correlation between anti-Ttd IgG titers in the serum of PBL donors and SCID mice. In 7 PBL donors, anti-Ttd IgG titer was undetectable. Among them, 6 donors were aged 40 or older. The number of cases in which anti-Ttd IgG was negative in SCID mice while positive in the PBL donor was 33 without immunization, and 19 with immunization. The number of cases in which anti-Ttd IgG titers in SCID mice were higher than those in the PBL donors were 4 and 29 without and with immunization, respectively.

Discussion

In this study, SCID mice were transplanted with human PBL obtained from 88 healthy donors, and anti-Ttd antibody production in these hu-PBL-SCID mice was investigated. hu-PBL-SCID mice produced anti-Ttd IgG antibody from 48 PBL donors without in vivo immunization and from 63 donors with in vivo immunization. The average anti-Ttd IgG titer was elevated significantly by the in vivo immunization. Both the ratio of positive anti-Ttd IgG antibody production and average antibody titer were significantly higher in mice with PBL transferred from donors under 40 than those in mice with PBL transferred from donors aged 40 or older (fig. 1). These donor age-dependent differences in anti-Ttd IgG production in hu-PBL-SCID mice might be attributable to Japan's vaccination program of diphtheria-pertussis-tetanus vaccine, which took place in 1968 [10]. Another interpretation of the donor age-dependent differences observed in this study is that the differences in anti-Ttd IgG antibody production in SCID mice might represent different vaccination stages, i.e. time since vaccination, of the PBL donors. However, information regarding individual donors is limited because the PBL used in this study were obtained from donated blood. Therefore, anti-Ttd antibody production in hu-PBL-SCID mice was thought to be dependent on the memory of tetanus in the transferred PBL.

Discrepancies in serum anti-Ttd IgG titers between PBL donors and SCID mice were observed (fig. 5). Nineteen cases were negative for anti-Ttd IgG production in the SCID mice, while anti-Ttd IgG were positively detected in the donor sera. However, it is unlikely that those cases in which anti-Ttd IgG antibody production was undetectable in the SCID mice were caused by a failure of human PBL repopulation in the mice since, in those cases, significant amounts of human Ig were detected in the sera

of SCID mice and, overall, the amounts of antigen-specific and total IgG did not necessarily correlate with each other (data not shown). It is also unlikely that a previous Epstein-Barr virus (EBV) infection in the donors affected anti-Ttd IgG production in the SCID mice. Among 88 donors, 52 (59%) were positive for EBV infection. However, no correlation was found between positive EBV infection in the PBL donors and antigen-specific IgG production in the SCID mice. In general, the large experimental variation, which is probably donor dependent [11], in the hu-PBL-SCID model is a known problem [12]. Perhaps the discrepancy in anti-Ttd IgG production between PBL donors and SCID mice found in this study could be due to donor-dependent variation, although its precise mechanism is unclear.

Complete protection against lethal doses of Ttx was observed in 14 cases without in vivo immunization with Ttd, and the number of cases was increased to 24 by in vivo immunization with Ttd (fig. 2), suggesting that the production of neutralizing antibody is enhanced by in vivo immunization. Among those groups of mice in which protection against Ttx was observed, 92% without in vivo immunization and 87% with in vivo immunization had received PBL from donors under the age of 40. These results suggest that people older than 40 are less protected against tetanus than is the younger population in Japan.

Interestingly, 3 cases were found in which mice were protected against challenge with Ttx although anti-Ttd IgG antibody was undetectable in the mice's sera (fig. 3a). It is unlikely that an Ig class other than IgG against Ttd

contributed to this protection, since Ttd-specific IgM, IgA and IgD were also undetectable in those serum samples (data not shown). Perhaps, in these cases, an undetectable level of neutralizing antibody effectively contributed to the protection. In fact, serum anti-Ttd IgG antibodies were positively detected in all these 3 cases in the groups of mice that received in vivo immunization and all those mice were protected against Ttx. In contrast, 2 cases were found in which a high level of anti-Ttd IgG antibody was detected in the sera of SCID mice although no protection against Ttx was observed (fig. 3b). These discrepancies between serum anti-Ttd IgG level and the protective ability against Ttx suggest that the amounts of antibody against Ttd and neutralizing antibody directed against Ttx [13] do not always correlate with each other. The discrepancy between specific antibody titer in the serum and protective activity against Ttx observed in hu-PBL-SCID mice might reflect the actual condition of the PBL donor. From this point of view, the assessment of vaccine efficacy would be more critical if vaccines were evaluated in terms of the induction of neutralizing antibody formation, and not by the induction of antigen-binding antibody production.

Thus, the results of this study suggest that the hu-PBL-SCID model is potentially applicable as a tool for predicting protective activity in PBL donors. Moreover, since the effects of in vivo immunization were observed in many cases, the hu-PBL-SCID model could possibly be applied for the evaluation of vaccine efficacy, provided that this system is refined in respect to reproducibility, by selecting PBL donors.

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