

Reduction of human anti-tetanus toxoid antibody in hu-PBL-SCID mice by immunodominant peptides of tetanus toxoid

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SUMMARY

Immunotherapy of murine autoimmune and allergic diseases by administration of peptides corresponding to the dominant T cell epitope is a reality. However, problems remain in applying this therapy to reduce antibody responses in humans. To overcome these difficulties, a preclinical system was developed to test the effect of immunodominant peptides from a common antigen, tetanus toxoid (TT), on the long-term human anti-TT response. Individuals whose T cells proliferated against dominant TT peptides were identified. Peripheral blood leucocytes (PBL) from these donors were injected intraperitoneally (i.p.) into mice with severe combined immunodeficiency (SCID) that had been depleted of murine natural killer (NK) cells (hu-PBL-SCID mice). Peptides or PBS were injected i.p. before a further injection of PBL and immunization with TT. The concentration of human IgG and anti-TT in murine plasma was followed for 10 weeks. The total IgG was similar in both groups. By contrast, there was a statistically significant reduction in IgG anti-TT from eight weeks onwards. It is considered that the hu-PBL-SCID model system may provide a means by which the efficacy of peptide immunotherapy for reduction of pathological antibodies in humans can be examined.

Keywords SCID hu-PBL-SCID peptides tetanus toxoid anti-tetanus toxoid IgG

INTRODUCTION

Antibodies are pathogenic in a number of alloimmune, autoimmune and allergic diseases. For example, anti-D produced by Rh-negative women used to be the leading cause of haemolytic disease of the fetus and newborn (Rhesus disease) until the incidence was dramatically reduced by the prevention of primary immunization with prophylactic anti-D [1]. A similar disease is caused by maternal immunization to fetal platelet antigens, usually the human platelet antigen (HPA)-1a, that can result in alloimmune thrombocytopenia of the fetus and newborn. Placental transfer of the antibodies from the mother to an HPA-1a-positive fetus may cause severe thrombocytopenia [2] in some cases resulting in intracranial haemorrhage [3]. At present, there is no prophylactic anti-HPA-1a [4]. Antibodies to HLA antigens are responsible for many cases of platelet refractoriness [5] and may cause early [6] or chronic [7,8] rejection of transplants. Some autoimmune diseases such as autoimmune haemolytic anaemia, autoimmune thrombocytopenia and myasthenia gravis are caused mainly by IgG autoantibodies. Atopy is due to production of IgE

antibodies to allergens. Immunosuppression with steroids and/or intravenous immunoglobulin is partially effective in many of these cases, but is nonspecific and may have side-effects [9–12]. Therefore an immunosuppressive treatment targeted to the particular pathogenic antibodies would be of great value in all these diseases.

One form of specifically manipulating immune responses that has proved effective in animal models is administration of peptides containing immunodominant T cell epitopes. For example, the myelin basic protein (MBP) peptides Ac1–11 and Ac1–9 prevented experimental allergic encephalomyelitis [13,14]. Diabetes in non obese diabetic (NOD) mice was decreased with glutamic acid decarboxylase peptides [15] or insulin peptide B 9–23 [16]. Pathogenic antibodies and disease in experimental autoimmune myasthenia gravis were reduced by administration of dominant peptides from the acetyl choline receptor 150–169 [17]. Similarly, in NZB mice with autoimmune haemolytic anaemia, a soluble analogue (E861, K865) of the dominant T cell epitope of red blood cell band 3 (peptide 861–874) modulated both the red blood cell autoantibody response and the disease. Interestingly, the native 861–874 peptide, which is relatively insoluble, promoted autoantibody development and anaemia [18]. Finally, antibody responses to the house dust mite allergen Der p 1 were reduced in sensitized mice by the Der p 1 111–139 peptide [19].

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The aim of the current work was to establish a model whereby the effect of peptides on long-term human antibody responses could be studied. Mice with severe combined immunodeficiency (SCID) lack T and B cells and readily accept transplantation of xenogeneic cells. They can be 'reconstituted' with a human immune system by injection of human peripheral blood leucocytes (PBL) [20], and can produce human IgG for at least three months postengraftment. We have previously used SCID mice to generate anti-A and anti-D responses to A⁺ or D⁺ human red blood cells [21,22] and to optimize human anti-tetanus toxoid (TT) antibody responses [23]. Here we report experiments designed to determine if SCID mice can be used to study the effect of immunodominant TT peptides on the human anti-TT response. TT was chosen both because of the ready availability of PBL from healthy donors immunized to this foreign antigen and because TT requires processing and presentation by APC [24], generating a T helper cell dependent immune response [25].

Three TT peptides containing immunodominant T cell epitopes have been identified already [26]. Two peptides (p30 and p2) elicited proliferative responses by T cells from the majority of primed donors despite the disparate HLA class II haplotypes of the individuals. By contrast, the peptide p4 was recognized by T cells from about half the donors in association with only DRw52a and DRw52c [25,26]. We used these peptides and TT to identify individuals with circulating antigen specific T cells by monitoring cellular responses in a T cell proliferation assay. We then tested the effect of these peptides on the total IgG and specific IgG anti-TT responses in SCID mice reconstituted with PBL from donors with TT-specific T cells. It was found that the IgG anti-TT response could be reduced and curtailed by peptide immunotherapy.

MATERIALS AND METHODS

Peripheral blood leucocytes (PBL)

Anticoagulated blood was donated by 31 healthy adults (aged 23–48 years) who had been vaccinated with TT 1–10 years previously. PBL were isolated by density gradient centrifugation over Histopaque-1077 (Sigma, Poole, UK) and washed thrice in RPMI 1640 medium (PAA Laboratories, Yeovil, UK) containing 10 iu/ml Heparin (CP Pharmaceuticals, Wrexham, UK), 100 IU/ml penicillin (PAA), 50 IU/ml streptomycin (PAA) and 25 IU/ml polymyxin (PAA).

Peptides and antigens

The 12-, 15- and 21-mer TT peptides were synthesized using Fmoc chemistry on resin with 85–95% purity tested by HPLC and amino acid analysis. The lyophilized peptides were stored at –30°C. Aliquots (3–4 mg) of each peptide were solubilized in dimethylsulphoxide (DMSO) (30 µl), diluted to a concentration of 1–3 mg/ml in sterile phosphate buffered saline (PBS) and 0.2 µm filtered. The amino acid sequences were:

p30 (947–967) F N N F T V S F W L R V P K V S A S H L E
p2 (830–844) Q Y I K A N S K F I G I T E L
p4 (1273–1284) G Q I G N D P N R D I L

Mycobacterium tuberculosis purified protein derivative (PPD) was obtained from the UK Veterinary Laboratory, Surrey. Purified tetanus toxoid (TT) was adsorbed tetanus vaccine supplied at 80 IU/ml (1 mg/ml) (Evans Medical Ltd, Leatherhead, UK) and tetanus toxin supplied at 6 mg/ml (Swiss Serum and Vaccine Institute, Berne, Switzerland).

T cell proliferation assay

PBL isolated from 25 to 40 ml blood were aliquotted at 2 ml/well into 24-well tissue culture plates (Nunc International, Costar, New York) at a concentration of 1.4×10^6 /ml in MEM containing antibiotics (as above) and 1 mM glutamine (HyQ, Cramlington, UK). Autologous serum was prepared by heating plasma at 56°C for 30 min followed by centrifugation at 2000 g for 10 min; 100 µl were added to the wells. Stock solutions of the antigens were made in PBS and aliquots added to the wells to give final concentrations of 50 µg/ml (PPD), 1–25 µg/ml (tetanus toxin) and 0.1–1 iu/ml (adsorbed tetanus vaccine). TT peptides were added at varying concentrations ranging from 1 to 25 µg/ml (1–30 µl per well). Control wells received an equal volume of MEM. The plates were incubated at 37°C in a humidified 5% CO₂ incubator for 7 days and duplicate aliquots of 100 µl cell suspension transferred into wells of a 96-well round bottomed plate on days 4–7. Then 16 µl ³H-thymidine (Amersham International, Amersham, UK) was added to each well (0.5 µCi/well) and the plates incubated as above for 16–24 h prior to harvesting the cells onto glass fibre filter mats (LKB-Wallac, Turku, Finland) using a Mach 111 harvester 96 (Tomtec, New Jersey, USA). Thymidine incorporation was determined using a Microbeta liquid scintillation counter (LKB-Wallac) [27]. The responses (cpm) of the test and control wells were expressed as the stimulation index, SI = cpm (test)/cpm (control). Values of 3 SI and over were considered a positive response. The cumulative SI was the total of the individual SI for the four days of sampling.

Mice

CB 17 SCID mice and NOD-SCID mice were bred and maintained in pathogen-free flexible film isolators. Blood samples were taken from the tail veins into heparinized capillary tubes (Richardsons, Leicester, UK) and the plasma tested for murine immunoglobulins (IgG and IgM) by enzyme-linked immunosorbent assay (ELISA) [28]. Mice between 6 and 12 weeks of age and with total Ig concentrations less than 10 µg/ml were used. Natural killer (NK) cells, which reduce graft acceptance [29] were depleted by injecting 12 µl anti-asialo GM-1 (Wako Chemicals, Wako, USA) intraperitoneally (i.p.) on days –1, 0 and 7. The antibody was diluted in 100 µl sterile PBS for the day –1 dose and was incorporated into PBL on day 0 and into TT peptides on day 7.

Injection of PBL, peptides and TT to SCID mice and blood sampling

All injections were i.p. Groups of 5–8 mice were used. PBL were injected on day 0 and in some experiments also on day 14; $10\text{--}30 \times 10^6$ cells in a volume of 500 µl sterile PBS were given to each mouse. The first dose of peptides, or PBS in control mice, was incorporated into the PBL on day 0, and thereafter the peptides (or PBS) were injected in a volume of 200 µl sterile PBS. Each dose of peptide was 200 µg. The mice were immunized with antigen (TT) on day 0 or days 14 and 21; the TT was incorporated into the PBL or given in 100 µl sterile PBS. Tail vein bleeds were taken as above at 2–4 week intervals to monitor the human antibody responses.

Quantification of human IgG and human IgG anti-TT by ELISA

Approximately 5–50 µl murine plasma was obtained at each time point and an initial dilution of 1/100 made in RPMI containing 10% fetal calf serum. The concentration of human IgG was

determined using affinity isolated goat anti-human IgG (Sigma) as capture and peroxidase conjugated reagents [28] and purified monoclonal human IgG1 and IgG3 anti-D [30] as standard (Bio Products Laboratory, Elstree, UK). The limit of detection was 0.2 µg/ml. The concentration of human IgG anti-TT was determined similarly except that the plates were coated with adsorbed TT at 2.2 µg/ml (0.16 iu/ml) in carbonate buffer, pH 9.6, and the standard was purified IgG anti-TT (Bio Products Laboratory) used at a range of concentrations between 0.001 and 10 iu/ml.

Human cytokine detection

The presence of human cytokines in the hu-PBL-SCID plasma was determined using the Cytometric Bead Array Kit from BD Pharmingen (Oxford, UK). Briefly, aliquots of capture bead suspensions, detection reagent and human cytokine standards (20–5000 pg/ml) or test samples (plasma diluted 1/10) were incubated together prior to analysis by flow cytometry according to the manufacturer's instructions. The assay sensitivity for the human cytokines IL-2, IL-4, IL-6, IL-10 and TNF-α was 3 pg/ml whilst for IFN-γ it was 7 pg/ml.

Statistical analysis

Significant differences in human antibody levels between control and treated mice were analysed using the Mann-Whitney *U*-test using GraphPad Instat version 4.0 for Windows 95 (GraphPad Software, San Diego, California, USA).

RESULTS

Preliminary experiments were conducted to determine whether the SCID model used in earlier experiments [23] could be improved by increasing the amount of human IgG produced and reducing the variability in IgG responses between individual mice. The effect of depleting SCID mice of NK cells and using NOD-SCID mice was examined. PBL from one donor were injected i.p. into SCID mice, SCID mice depleted of NK cells and NOD-SCID mice (17×10^6 PBL/mouse) together with 5 µg TT. Human antibody concentrations were measured in murine plasma at intervals from 2 to 8 weeks. SCID mice depleted of NK cells produced by far the most IgG and IgG anti-TT (Fig. 1) over the two month experimental period and had less intragroup variability in IgG levels than untreated SCID mice or NOD-SCID

mice. SCID mice depleted of NK cells were therefore used for the remainder of the experiments.

Selection of donors

It was necessary to select donors whose T cells responded to the dominant TT peptides. Accordingly, PBL were prepared and their ability to proliferate *in vitro* to PPD, TT and the TT peptides was examined. Of 31 individuals tested, all reacted with PPD and 77% with TT. The responses to TT were greater than those to the peptides and the latter elicited more variability in responses (both in magnitude and kinetics) over the period of culture. The peptides were tested at concentrations from 1 to 25 µg/ml; no single concentration was optimal. The percentage of donors (and of 44 samples) exhibiting proliferative responses to p30 was 52% (44%), to p2 was 52% (37%) and to p4 was 60% (47%). Repeat samples from donors also gave some variability in responses to all the antigens. From these results, four donors were chosen for the final mouse experiments because their T cells responded to at least one of the peptides used (Fig. 2). As can be seen from Fig. 2, T cells from donors A, C and D proliferated to all three peptides while only p30 elicited a response from the T cells of donor B.

Human antibody responses in hu-PBL-SCID mice

In eight experiments, antibody concentrations were measured in murine plasma taken at intervals up to 3 months from hu-PBL-SCID mice that were injected with PBL from the four selected donors and immunized with TT on day 0 or days 14 and 21. After injection of PBL, little IgG was detected by 2 weeks but then it increased steadily for several weeks, reaching maximum concentrations in murine plasma at 7–10 weeks, with up to 3 mg IgG per ml in some samples of murine plasma. Not all mice showed evidence of 'reconstitution', only 80–90% of mice produced human IgG; those that did not were excluded from further analysis. IgG anti-TT responses peaked at 6–8 weeks, slightly earlier than the total IgG. Anti-TT concentrations in the murine plasma ranged up to 12 iu/ml and in some samples were similar to those in the plasma of the donors (5–10 iu/ml).

Effect of peptides on human antibody responses in hu-PBL-SCID mice

Four initial experiments resulted in similar anti-TT levels in SCID mice injected with various doses of PBL, TT and TT peptides at different times. For these experiments, mice were given

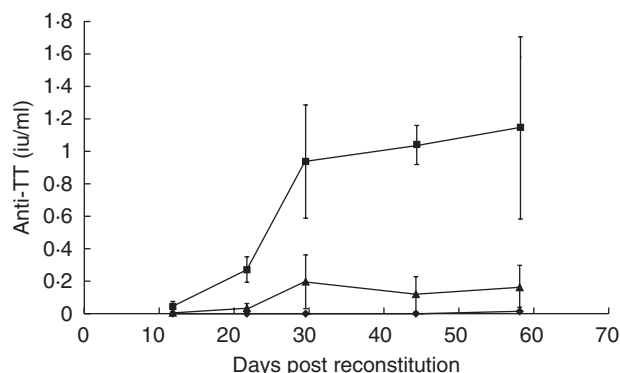


Fig. 1. Comparison of human IgG anti-TT concentrations in plasma of mice injected with 17×10^6 PBL on day 0. SCID mice injected with GM-1 antibody to deplete NK cells (■); NOD-SCID mice (▲); SCID mice (◆). Mean $\pm$ SEM.

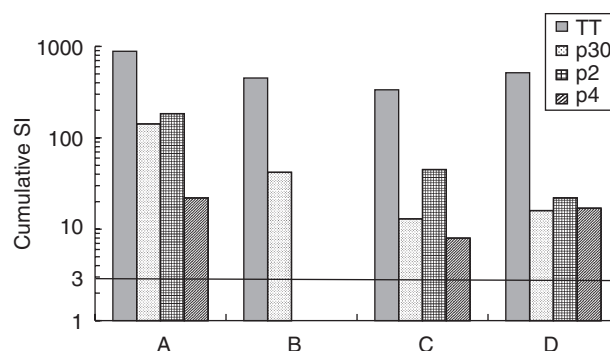


Fig. 2. *In vitro* T cell proliferative responses of four donors (A, B, C and D) to tetanus toxoid (■), TT peptide p30 (▨), TT peptide p2 (▩) and TT peptide p4 (▤). The cumulative SI (summation of individual SI of days 4–7) is shown. The concentration of the peptides was 10 µg/ml.

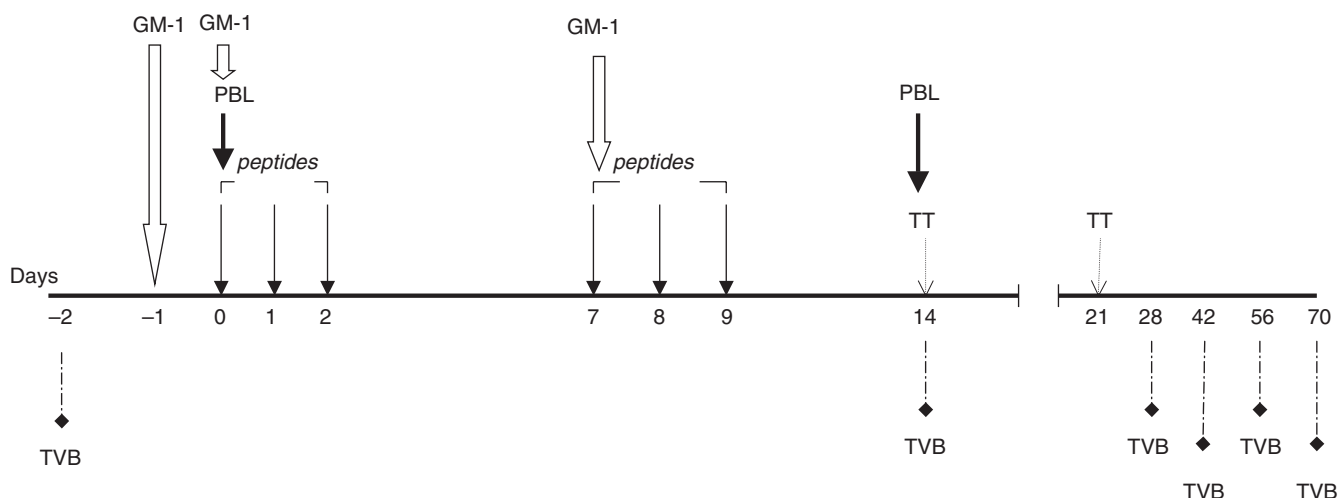


Fig. 3. Scheme of study in SCID mice. Plasma was obtained by tail vein bleeding (TVB) in prestudy samples and tested for murine Ig. All injections were intraperitoneal. GM-1 antibody was injected on days -1, 0 and 7 to deplete murine NK cells. PBL were injected on days 0 and 14; each mouse received PBL from one donor, at doses indicated in the text. Peptides were administered on days 0, 1, 2, 7, 8 and 9; a total dose of 600 μg consisting of 200 μg each of p30, p2 and p4 was given at each injection. Tetanus toxoid (1 μg or 1 iu) was injected on days 14 and 21. Mice were bled every two weeks by tail vein bleeding (TVB) for assay of human antibodies.

20–30 $\times 10^6$ PBL/mouse in a single injection on day 0, TT on day 0 (in one experiment at days 15 and 21) and TT peptides on days 3, 6, 10 or 18 at a total cumulative dose of 300–6000 μg . It was thought that any regulatory effect of the peptides *in vivo* might be counteracted by the early immunizing stimulus of TT and that any suppressive activity induced might be better detected if PBL were first exposed to peptides and measurements made of their ability to confer unresponsiveness on a second dose of PBL. Accordingly, a different protocol was employed (Fig. 3). PBL were injected into SCID mice and the mice injected with the peptides. Two weeks later the mice were given another dose of PBL harvested from a fresh blood donation from the same donor, immunized with TT and the total IgG and anti-TT concentrations measured at intervals thereafter. Four experiments were set up, using cells from each of the donors selected.

The results from donors A, B and C are summarized in Figs 4 and 5. These mice received a total of 45×10^6 , 52×10^6 and 58×10^6 PBL/mouse from these donors, respectively, and had high concentrations of human IgG. Variability in the dose of PBL used was due to differences in the number of PBL in the donors' blood. Mice given PBL from donor D (a total of only 23×10^6 PBL/mouse) had low human IgG concentrations (maximum of 0.7 mg/ml), 2–3 times less than that of mice receiving PBL from the other donors, with no difference between peptide treated and control groups. With donors A, B and C there was little difference in total IgG concentrations between the peptide-treated and control groups although the former had usually slightly lower mean concentrations (Fig. 4). At one sampling point a significant difference was observed (day 70, $P = 0.014$, Fig. 4b) but this difference was not maintained (day 110, $P = 0.73$, data not shown). When the data from the three experiments was combined there was no significant difference in total IgG concentrations at any time point (Fig. 6a). In contrast, the anti-TT concentrations in peptide-treated mice appeared less than in the controls. For donor B (Fig. 5b) the difference was significant at day 42 ($P = 0.021$), day 56 ($P = 0.014$) and day 70 ($P = 0.002$), and also at day 110

($P = 0.029$, result not shown). For the other two experiments, the difference at day 70 (Fig. 5a) and day 56 (Fig. 5c) did not quite achieve significance ($P = 0.063$ and $P = 0.059$, respectively). Combining the data from the three experiments showed the maximum anti-TT response peaked earlier and then declined more rapidly in the peptide-treated groups than in the control groups (Fig. 6b). Although specific antibody levels were similar for the first month, and did not differ significantly on day 42 ($P = 0.99$), by the latter part of the experiment the mean anti-TT concentration in the plasma of peptide-treated mice was much lower than that of the control mice, and at day 56 ($P = 0.002$) and day 70 ($P = 0.0005$) the differences were significant (Fig. 6b).

Human cytokines in hu-PBL-SCID mice

None of four samples, taken on day 56 from mice given donor A or B PBL, with or without peptides, had detectable IL-2, IL-4, IL-6, IL-10, TNF- α or IFN- γ . Insufficient plasma remained from the other samples after assaying for total IgG and anti-TT to test for these cytokines.

DISCUSSION

SCID mice depleted of NK cells were found to be the most suitable murine model for our study of the long-term human IgG responses after i.p. injection of human PBL. The mice remained healthy throughout the term of the experiment and engrafted well. The number of PBL transplanted was kept below the level at which mortality due to tumours or graft-versus-host disease might develop [21,23,31,32] and these problems were not encountered during this investigation. The half life of human IgG in SCID mice was documented as 10–12 days [33]. Thus the sustained human IgG response in the current study indicated that human B cells survived and proliferated in SCID mice for several months. Previous work suggests that the hu-PBL-SCID mouse model is versatile and it has been used for manipulating human antibody responses. For example autoantibody production required the

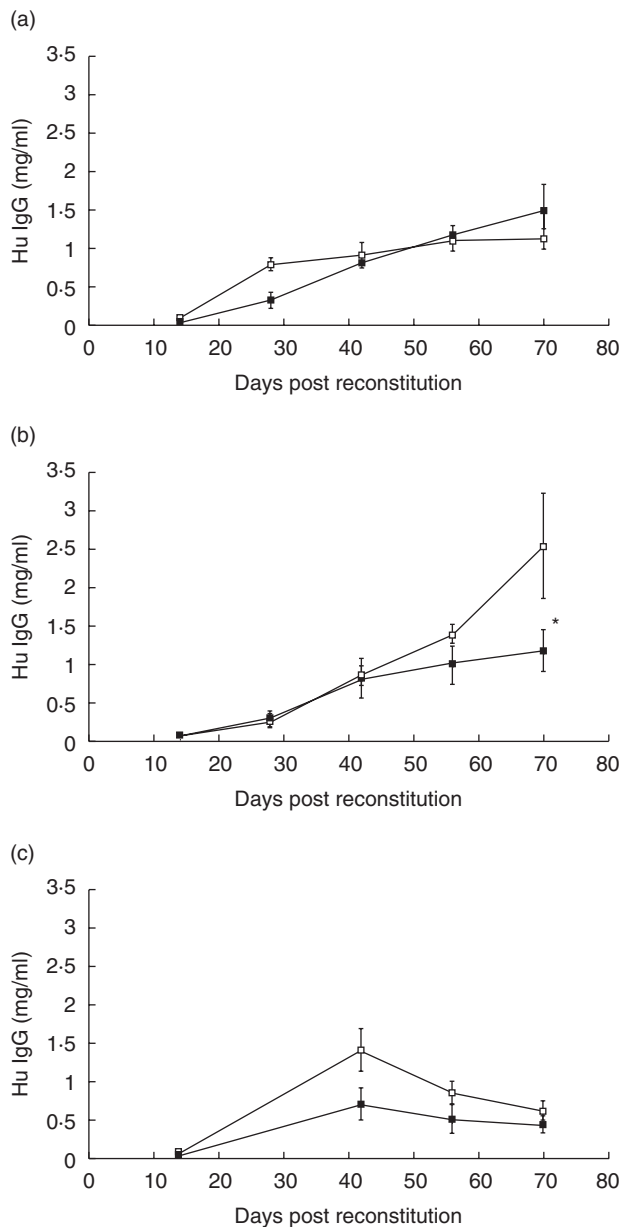


Fig. 4. Total human IgG concentrations in plasma of SCID mice injected and bled according to the protocol illustrated in Fig. 3. Mice were given (a) 27×10^6 and 18×10^6 PBL from donor A, (b) 31×10^6 and 21×10^6 PBL from donor B or (c) 27×10^6 and 31×10^6 PBL from donor C on days 0 and 14. Mice were also injected with PBS ($\square$) or peptides ($\blacksquare$). * $P < 0.05$.

presence of CD4⁺ T cells [34,35] and synthesis of alloantibodies was dependent on functional interactions between CD4⁺ T cells and B cells [36].

Our study showed that it was possible to reduce the specific antibody response to TT by administration of three soluble immunodominant peptides of TT. SCID mice were used to maintain the viability of human leucocytes for over three months. Various experimental strategies were initially taken. From this, it was concluded that the peptides only reduced anti-TT responses if (a) each mouse received more than 40×10^6 PBL (b) two doses of PBL were injected, two weeks apart and (c) the immunization with TT was delayed until after the initial dose of PBL and

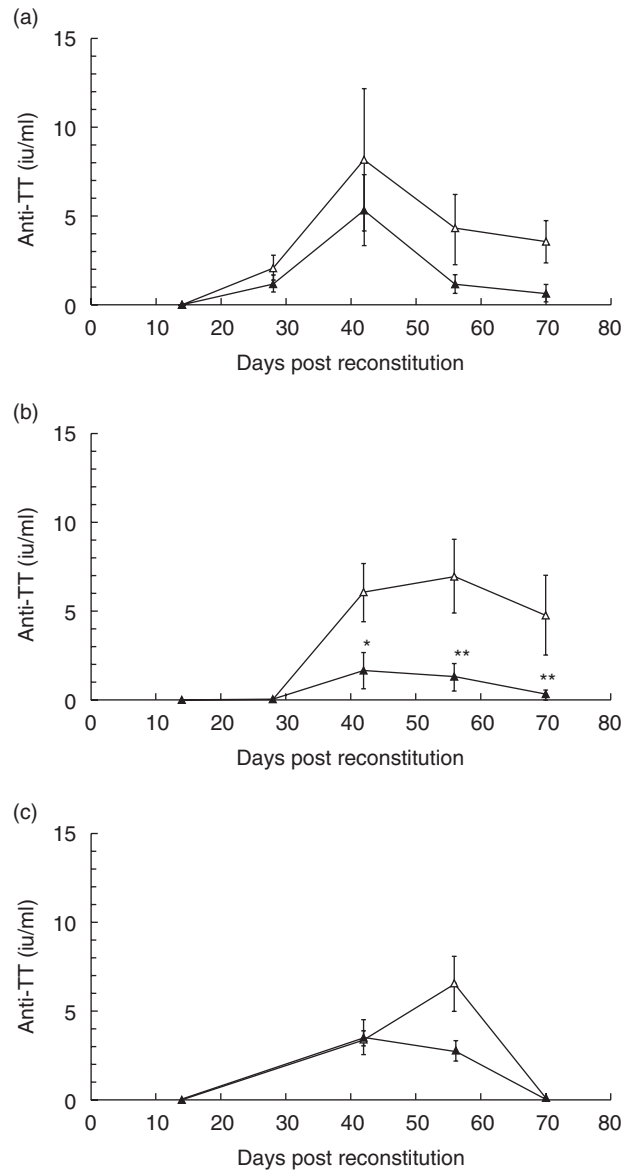


Fig. 5. Human IgG anti-TT concentrations in plasma of SCID mice injected and bled according to the protocol illustrated in Fig. 3. Mice were given (a) 27×10^6 and 18×10^6 PBL from donor A, (b) 31×10^6 and 21×10^6 PBL from donor B or (c) 27×10^6 and 31×10^6 PBL from donor C on days 0 and 14. Mice were also injected with PBS ($\triangle$) or peptides ($\blacktriangle$). * $P < 0.05$, ** $P < 0.01$.

peptides. Our interpretation of the latter two conclusions is that they are consistent with the peptides stimulating some form of regulatory cell.

The precise method by which the peptides modulated the anti-TT response is not yet clear. However, it is known that peptide-induced tolerance of experimental autoimmune encephalomyelitis is dependent on IL-10 secreting T cells [37,38]. (Here an attempt was made to detect human IL-10 in hu-PBL-SCID plasma but none was detected presumably because of the relatively low numbers of human cells in the mice). It appears that induction of IL-10 secreting T cells is favoured by contact with immature dendritic cells. For example, in humans, suppressive antigen-specific CD4⁺ [39] or CD8⁺ [40] T cells are generated by repeated

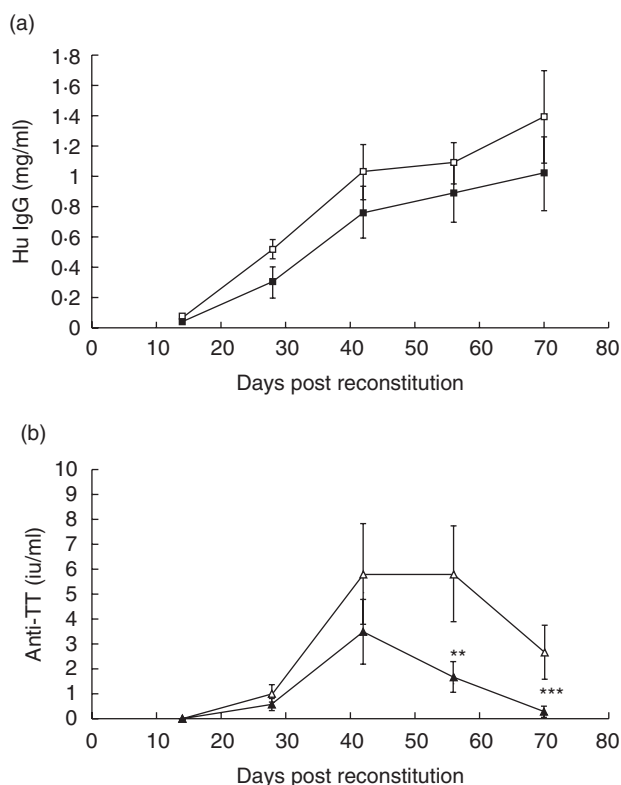


Fig. 6. Combined data from the three experiments illustrated in Figs 4 and 5. Total human IgG (a) and human IgG anti-TT (b). The mean $\pm$ SEM are shown for mice injected with PBS ($\square$, $\triangle$) or peptides ($\blacksquare$, $\blacktriangle$). ** $P < 0.01$, *** $P < 0.001$.

stimulation with immature DCs. The SCID mouse environment would be void of inflammatory stimuli and therefore human dendritic cells from the injected PBL might remain in an immature form. These DC express empty or peptide-receptive HLA class II molecules at the cell surface, so that peptide loading may occur extracellularly [41]. Peptides with high affinity for HLA class II may therefore have bound directly at the cell surface of the DC. Notably, the immunodominant p30 and p2 peptides form very stable, irreversible, complexes with DR11 [42]. If there were no intracellular activation signals in the DC or up-regulation of costimulatory molecules, the T cells whose T cell receptor bound the peptide-HLA class II complex would not be activated but rather modulated leading to their suppression or anergy. Low B7 (CD80) on immature DC would result in preferential engagement of CTLA-4 on T cells favouring the induction of T regulatory cells. Induction of such cells (T cell tolerance) is known to be associated with an increase in the proportion of CTLA-4⁺ cells [38]. T regulatory cells would hinder Th cells from providing the necessary signals required to stimulate the appropriate TT primed B cells into antibody production. If so the effect appears long lasting.

The anti-TT responses were initially similar in the peptide-treated and control groups of mice, and only diverged after about six weeks. From this time onwards curtailment of anti-TT antibody response was observed in the TT peptide treated mice.

It is interesting that the i.p. route of peptide administration was effective in our hu-PBL-SCID model. In other studies, inhalation of peptides reduced disease in murine models of autoimmunity and allergy. An explanation for this may be because the

normal mucosa is naturally tolerant of most antigens. However, since TT and most/all alloantigens are encountered systemically or intravenously and localize to the peripheral or draining lymph nodes or spleen, perhaps the intranasal route of peptide administration would not be so appropriate for these antigens.

The nature of peptides appears to affect their ability to suppress or induce immune responses. For inhibition of autoimmune haemolytic anaemia, only a soluble analogue of the relatively insoluble native immunodominant peptide was beneficial [18]. In another system high affinity peptides were more efficient in tolerance induction [43]. Dominant T cell epitopes of antigens are often identified by their capacity to stimulate T cell proliferation *in vitro*. However, this assay may not reflect their mode of action *in vivo*. *In vitro*, the APC become spontaneously activated by adherence to the plastic culture plates, so that presentation of the bound peptide may always be in a stimulatory context whereas *in vivo* in experimental mice they may remain immature. It is notable that in our study there appeared to be little relationship between the extent of T cell proliferation towards the TT peptides *in vitro* and suppression of the anti-TT response by these peptides *in vivo*. It would appear therefore that peptides stimulating T cells *in vitro* may vary with respect to their inhibitory properties *in vivo*. In this study we observed that *in vitro* reactivity of peripheral blood T cells to PPD, TT and peptides of TT varied over time, independently. Such fluctuation in T cell responses in repeat samples from donors has been noted before. This occurred with samples taken at approximately monthly intervals from patients with multiple sclerosis (MS) responding to myelin proteolipid protein (PLP) peptides [44]. Similarly, reactivity to MBP peptides showed considerable diversity when blood was taken at 3–10 month intervals from MS patients [45]. The response of healthy subjects and MS patients to TT also varied greatly over 1–2-month periods [44]. Lesser differences in proliferation to TT peptides were observed at two week intervals [46]. This phenomenon may reflect the frequency of antigen-specific T cells in the peripheral circulation due to cyclical re-activation.

It may be more difficult to reduce antibody responses to a foreign protein, such as TT, than to non-MHC alloantigens. The former has many T cell epitopes, from different sequences of the protein [46,47] with variability in the immunodominant peptides between different individuals due to MHC restriction. Some of these peptides may exhibit promiscuous binding to MHC class II and recognition by T cells [26]. In contrast, peptides from an alloantigen are likely to be immunogenic only if they encompass a residue change of the polymorphism. This may account for the high MHC restriction for alloantibody production in some diseases, most notably DRB3*0101 (DR52a) in neonatal alloimmune thrombocytopenia due to anti-HPA-1a [48].

The observation here that immunodominant TT peptides could specifically diminish and curtail the human anti-TT response *in vivo* suggests that the Hu-PBL-SCID system may be of value in testing peptide immunotherapy for the control of such diseases.

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