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Immunosuppression by Fc region-mismatched anti-T cell antibody treatment

Formation of anti-antibodies (anti-Ab) is known to counteract immunotherapy with anti-T cell antibodies. Our previously described immunological approach prevented anti-Ab with the consequence of prolonged survival of fully mismatched skin grafts in C57BL/6 mice. These mice were treated with a single priming injection of a monoclonal anti-T cell Ab followed by repeated injections of anti-T cell mAb differing in species origin from the priming mAb. We now show prolonged tolerance to discordant xenogeneic, to bispecific, and even to polyclonal Ab, and demonstrate that the underlying immunosuppressive principle is due to a difference in heavy chain constant region between first and second antibodies, independent of whether or not they share the same idiotype. To examine this phenomenon, a panel of mAb was generated which share the same mouse anti-Thy-1.2 idiotype, but carry a human IgG1(T23), IgG3(T212C8), or mouse IgG2a(MmT1) constant heavy chain region. We found that sequential injection of MmT1 and T23 according to the above treatment schedule induced huIgG1 isotype-specific tolerance to T23, which was similar to that seen when using a primary mAb (MmT5) that was, instead, fully mismatched with T23 in both idiotype and constant region. Thus, differences of idiotype between primary and booster Ab were inconsequential for their ability to inhibit anti-Ab formation. This novel form of induced specific tolerance to anti-T cell Ig survived graft rejection and was still evident 230 days after termination of the T cell depletion protocol. Taken together, these results demonstrate that rechallenge with Fc region-mismatched Ab opens an immunological window that allows for induction of tolerance to immunogenic anti-T cell Ab and prolonged immunosuppression.

1 Introduction

Induction of inhibitory anti-antibodies (anti-Ab) [1–4] has been shown to limit monoclonal anti-T cell immunotherapy. Even humanized anti-T cell mAb have already been reported to induce anti-idiotypic Ab following a single course of treatment with humanized anti-T cell mAb [5]. We are interested in prolonging skin graft survival in fully mismatched C57BL/6 mice, a difficult model where peripheral T cell tolerance has heretofore not been reported. Using this model, we demonstrated that mice treated chronically with xeno- or even with allogeneic anti-T cell mAb rapidly developed anti-Ab, even during the first course of treatment, and showed graft rejection in the first month [6]. We used this immunization protocol to devise a method to prevent the formation of anti-Ab, consisting of sequential injections of anti-T cell mAb of two different species (mouse and rat), resulting in considerable prolongation of graft survival [6]. By generating recombinant anti-T cell mAb, we now show that the anti-Ab preventing mechanism is directly related to Fc region differences between the two injected anti-T cell Ab. Hence, anti-idiotypic Ab are suppressed, even though the sequentially applied mAb can share the same idiotype. A mouse predepleted of its $CD4^+$ T helper cells by the singly injected first mAb becomes tolerant to the multiply injected Fc region-mismatched second anti-T cell Ig, even if this second Ab is a discordant xenogeneic Ab, a bispecific quadroma derived Ab, or polyclonal anti-T cell Ig, like rabbit anti-mouse thymocyte globulin (RbATG). This latter finding is of special interest, since no Ab technologies are presently available for species adaptation of polyclonal Ab.

2 Materials and methods

2.1 Animals

C57BL/6 ($H2^b$) mice originally obtained from the Jackson Laboratory (Bar Harbor, ME) were raised and maintained in our breeding facilities. CBA ($H2^k$) mice were bred from stock in our laboratory. For transplantation experiments, 2–3-month-old female animals were used.

2.2 Antibodies

The following mAb were used for treatment *in vivo*: RmT1 (rat IgG2b anti-Thy-1) [7], RmCD4.2 (rat IgG2b anti-L3T4) [8], RmCD4.4 (rat IgG2a anti-L3T4) [8], RmCD8.2 (rat IgG2b anti-Ly2) [8], RmCD8.5 (rat IgG2a anti-Ly2) [8], MmT1 (mouse IgG2a anti-Thy-1.2) [9], MmT5 (mouse IgG2a anti-Thy-1.2) [9], T23 (chimeric human IgG1 MmT1), T212C8 (chimeric human IgG3 MmT1), G2 (bispecific 17A2 rat IgG2b anti-CD3 [10] × MmT1), BiB (bi-

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Abbreviations: anti-ab: Anti-antibodies **RbATG:** Rabbit anti-mouse thymocyte globulin

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specific RmCD8.6 rat IgG2a anti-Ly2 × MmT1) [11], RbATG (rabbit anti-mouse thymocyte globulin). mAb were isolated from culture supernatant by Protein G chromatography (Pharmacia, Freiburg, Germany). The binding activity of each purified mAb was checked by flow cytometry [12] or ELISA [6]. The quadromas G2 and BiB were produced as described [11, 13]. Purification to real bispecificity, excluding parental species, was performed on Protein A using pH gradient elution as described [11]. Recombinant Ab were generated by homologous recombination at the IgH locus of the hybridoma. Integration vectors were constructed [14] that contained the human C gene segments, a murine 5' homology flank and the *gpt* selection marker. Human (hu) IgG1 or huIgG3-containing plasmids pSV-gpt-huγ1-A4 and pSV-gpt-huγ3-A4, respectively, were linearized within the homology region and transferred into the anti-Thy-1.2 hybridoma MmT1 by electroporation [14]. Targeted recombinants were identified by ELISA using goat anti-human IgGFc as capture as well as detection Ab (Dianova, Hamburg, Germany).

2.3 Treatment protocols

Groups of six to eight C57BL/6 recipient mice were pre-injected i.p. with a single 400 μg dose (first Ab) of MmT1 or MmT5 mAb 3 days prior to transplantation of CBA/J skin grafts [6]. Chimeric T23 (second Ab) (200 μg), which carries the MmT1 idiotype and the human IgG1 C domains, was then injected i.p. beginning with skin grafting and continued twice a week until rejection. In other groups of mice, the following combinations of first and second Ab were used: T23/RmCD4.2+RmCD8.2, RmCD4.2 + RmCD8.2/T23, T23/RbATG, and RmCD4.2+ RmCD8.2/RbATG. In control mice, the pre-injection step was either omitted or the first and second Ab were identical. Tail blood samples for measurements of anti-Ab were collected 7–10 days after graft rejection. For studies of long-term tolerance to the second Ab, two protocols were used. First, C57BL/6 mice, pre-injected with MmT1 or MmT5 followed by chimeric T23 (Fig. 1A), were rechallenged 130, 144, 151 and 230, 244, 251 days after graft rejection with 20 μg per dose of T23 (second Ab), or RmT1 (xenogeneic "third party") or T212C8 (isotype mismatched "third party" chimeric Ab sharing the same idiotype with T23). Alternatively, C57BL/6 mice were pre-injected i.p. on day -3 with 500 μg T23, then injected with 200 μg RmCD4.2 + RmCD8.2 or RbATG (second Ab) on days 0, 4, 7, 11, 14, 21, 26, 30 and after an interval of 4 weeks, rechallenged i.p. with 20 μg of the second Ab on days 57, 69 and 78. Tail blood samples for measurement of anti-Ab were collected 7 and 14 days after the last injection of each rechallenge course. Inhibition of anti-Ab response to bispecific anti-T cell mAb was studied in C57BL/6 mice injected i.p. on day -3 with 400 μg T23 and 100 μg G2 or 50 μg BiB on days 0, 14 and 21, and bled 7 days later.

2.4 Quantitation of murine anti-Ab responses

Concentrations of mouse anti-Ig Ab were assessed by solid-phase ELISA as described [6]. In brief, microtiter plates were coated with mAb (MmT1, T23, T212C8, RmT1, RmCD4+CD8, RbATG) or (in some experiments) isotype-matched irrelevant controls. Serially diluted sera from treated animals or untreated controls or concentration standards (IDIO-1), mouse IgG1 anti-MmT1 idiotype [9], mouse anti-rat Ig (Dianova), or mouse anti-rabbit IgG (Dianova) were added and anti-Ab were detected using peroxidase-labeled goat anti-mouse IgGFc (Dianova), absorbed with MmT1 coupled to Tresyl-activated Sepharose 4B (Pharmacia), peroxidase-labeled rat anti-mouse IgG (Dianova), or peroxidase-labeled rabbit anti-mouse IgG (Dako, Hamburg, Germany). For studies of anti-bispecific Ab responses, microtiter plates were coated with parental mAb, and anti-Ab in serially diluted sera were detected using goat anti-mouse IgG-Fc absorbed with MmT1 or rat anti-mouse IgG.

3 Results

3.1 Suppression of anti-Ab response and prolonged skin graft survival after sequential treatment with Ab sharing idiotype but differing in Fc region

We observed [6] suppressed anti-Ab responses and prolonged skin graft survival following sequential treatment with two anti-T cell Ab differing in species origin. To determine whether the species differences between the C regions of the first and second Ab are sufficient for the observed immunosuppression/tolerance to the second Ab, we constructed anti-Thy-1.2 mAb sharing the mouse V regions but chimerized in the C regions. To this end, the human IgG1 or IgG3 isotype was introduced into the functional H chain locus of the hybridoma MmT1 by homologous recombination [14]. By sequencing the V_H and the Vκ gene from targeted transformants, we showed that homologous recombination is not mutagenic [14]. In addition, the MmT1 idiotype was also found to be preserved in that it is still able to bind to the anti-MmT1 idiotype Ab (IDIO1).

C57BL/6 recipients were injected i.p. with a single 400 μg dose of MmT1 3 days prior to transplantation of minor and MHC-mismatched CBA skin grafts. Flow cytometric analysis at this time point revealed strong depletion of blood T cells. $CD3^+CD4^+$ cells were reduced to 0.5 ± 0.25% (untreated control 13.1 ± 1.2%), $CD3^+CD8^+$ were reduced to 0.11 ± 0.09% (untreated control 12.1 ± 1.7%). Chimeric T23 (200 μg) sharing the V regions with MmT1 but carrying the human IgG1 C_H domains was injected i.p. on the day of skin grafting and continued twice weekly until rejection. As shown in Fig. 1A, 50% graft survival of up to 90 days occurred in MmT1/T23 (idiotype matched)-treated mice compared to about 20 days in mice treated with MmT1 or T23 only. Interestingly, mismatch in both idiotype and Fc-fragment between the first and second Ab was equally effective. As shown in Fig. 1A, pre-injection of MmT5 (mouse IgG2a anti-mouse Thy-1.2 originated from the same fusion as MmT1 but differing in idiotype) followed by T23 resulted in comparable graft survival as in the MmT1/T23 combination. T cell depletion caused by MmT1 and MmT5 were comparable. In agreement with improved graft survival, anti-Ab analysis (Fig. 1B) revealed almost complete suppression of the anti-second (T23) Ab response, regardless of whether or not the first and the second Ab shared common V regions. Evidently, species differences in the C regions between the pre-injected and booster Ab are sufficient for suppression of immunoresponse to the second Ab.

Specificity and durability of this hyporesponsiveness were further tested in mice treated with MmT1/T23 or MmT5/T23 (see above) by rechallenge with 20 μg T23 i.p. on days 130, 144, 151 and 230, 244, 251 after graft rejection and termination of T23. At these time points, T cell counts were found to be within normal ranges: blood lymphocyte counts by FACS analysis on day 100 in MmT5/T23-treated animals reached values of 17.3 ± 3.0% for $CD3^+CD4^+$, 10.4 ± 1.7% for $CD3^+CD8^+$ and 53.8 ± 5.0% for $B220^+$ cells; in MmT1/T23-injected mice, the values were 12.2 ± 3.5%, 8.8 ± 2.3% and 53.8 ± 6.1%, respectively. Control values in untreated mice were $CD3^+CD4^+$, 14.0 ± 2.2%; $CD3^+CD8^+$, 11.6 ± 1.7%; and $B220^+$, 54.0 ± 5.1%. The anti-T23 response was monitored 7 and 14 days after the last injection of each rechallenge sequence. As shown in Fig. 1C, four of six mice showed no anti-T23 response after the first rechallenge course; the other two mice showed only minute amounts of anti-T23 Ab. Conversely, a strong anti-third-party Ig response to RmT1 (rat IgG2b anti-mouse-Thy-1) with titers of 1/192 to 1/12288 was detected in mice that had not been rechallenged with T23. After the second course of rechallenge, on days 230, 244, 251, only minute amounts of anti-T23 Ab were detected in three of five mice; two other mice which had already responded to the first rechallenge attained anti-T23 titers of 1/144 and 1/4608 (Fig. 1C). This hyporesponsiveness was isotype-specific, as was confirmed (Fig. 1C) by control boosts with chimeric anti-Thy-1.2 (T212C8) sharing with T23 (and MmT1) the κ chain and V_H domain, but differing in isotype (huIgG3).

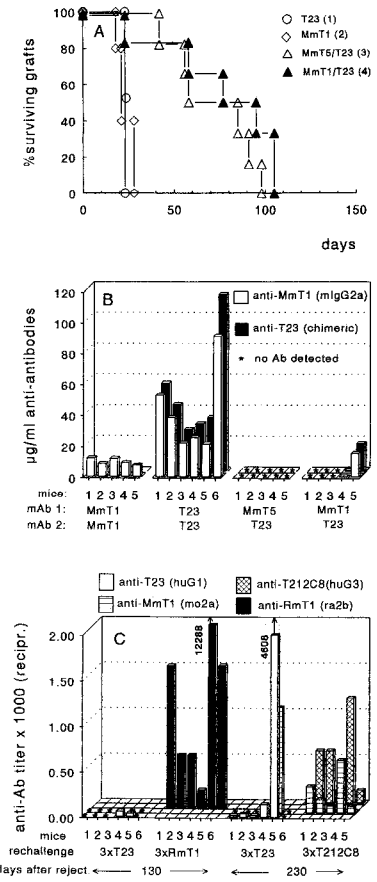

Figure 1. Xenogeneic C region mismatch between two sequentially injected anti-T cell mAb prevents anti-Ab response and prolongs graft survival. (A) Groups of six C57BL/6 mice grafted with fully mismatched CBA skin received one first Ab dose on day -3 (MmT1 or MmT5, same isotype, different idiotype) followed by twice weekly second Ab injections of chimeric T23 (MmT1 idiotype, human IgG1 isotype). Control animals received MmT1 or T23 mAb only. Loog-rank analysis of the survival curves gave the following *p* values of statistical significance: 1 *vs.* 2 = not significant; 1 *vs.* 3 = 0.0238; 1 *vs.* 4 = 0.0347; 3 *vs.* 4 = not significant. (B) Heparinized tail blood samples were collected 7–10 days after rejection (see A) and concentrations of anti-Ab were quantitated by ELISA. (C) Long lasting, isotype-specific Ab tolerance in mice treated as described in (A) and rechallenged with T23 (second Ab), RmT1 or T212C8 (third party Ab) as indicated. Titer of anti-Ig was revealed by ELISA and defined as serum dilution with an absorbance of 2 × mouse normal control.

The T cell-depleting capacity of the pre-injected first Ab seems to play a crucial role for the hyporesponsiveness against the second Ab. In C3H mice, where the first Ab (MmT1) showed only moderate T cell depletion in lymph nodes (19.8% for $CD3^+CD4^+$ and 4.3% for $CD3^+CD8^+$, 3 days after injection of 400 μg of MmT1; untreated controls, 47% and 23.4%, respectively), the hyporesponsiveness against the second Ab (RmT1, rat IgG2b anti-Thy-1) was only marginal (not shown). In contrast, the same Ab combination injected in C57BL/6 mice resulted in almost complete suppression of anti-second Ab response [6]. In this strain, MmT1 was highly T cell depleting (lymph node: 6.4% of $CD3^+CD4^+$, 3.1% of $CD3^+CD8^+$; untreated controls, 30.8% and 25.2%, respectively).

3.2 Anti-Ig response/tolerance and skin graft survival after sequential treatment with mAb and polyclonal RbATG

Of interest was whether preinjection of mAb would also suppress the immune response to polyclonal anti-T cell xenoantibodies. Such a combination would be especially relevant for potential human applications. For this reason, we injected 400 μg of rat RmCD4.2+CD8.2 mAb in C57BL/6 mice on day -3 followed by 600 μg RbATG (rabbit anti-AKR thymocyte globulin) on the day of CBA skin grafting and twice weekly until rejection. As shown in Fig. 2A, 100% of grafts survived longer than 150 days. Anti-Ab responses analyzed on day 160 revealed no anti-RbATG Ab. Similarly, after preinjection of chimeric T23 followed by RbATG, 50% of mice carried skin grafts longer than 150 days and again no anti-RbATG Ab were detected (Fig. 2A). In contrast, continuous treatment of the control groups with rabbit ATG or chimeric human T23 or rat RmCD4.2+RmCD8.2 without preinjection step resulted in only a 20–30 day prolongation of allograft survival (Fig. 2A). Consistently, strong anti-Ig Ab concentrations to RbATG (367.5 ± 171.7 μg/ml), T23 (50.8 ± 32.8 μg/ml) or RmCD4.2+RmCD8.2 (102.1 ± 62.0 μg/ml) were detected.

To compare our RbATG results with mAb treatments, we used also the CD4.2+CD8.2/T23 or the reciprocal T23/CD4.2+CD8.2 combination. As shown in Fig. 2A, over 150 days, 50% graft survival was observed in groups preinjected with rat RmCD4.2+CD8.2 followed by chimeric T23. In these mice, no anti-T23-Ab could be detected. Similar results were also observed using the inverse protocol. *i.e.* pre-injecting T23 followed by RmCD4.2+RmCD8.2 (Fig. 2A).

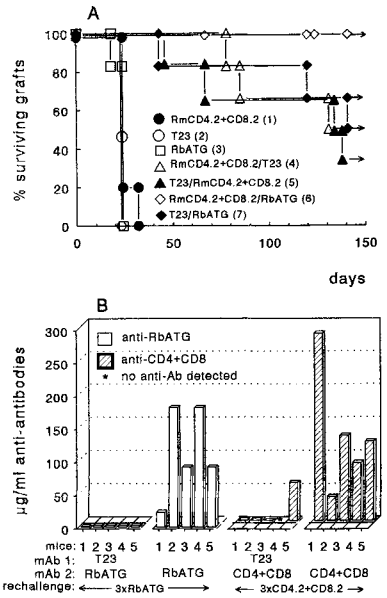

Figure 2. Prolonged allograft survival and tolerance to immunglobulin in mice treated with mono- or polyclonal anti-T cell Ab. First and second Ab were xenogeneic to each other and to the Ab recipient. (A) Groups of six C57BL/6 mice were pre-injected with T23 or RmCD4.2+CD8.2 (first Ab) 3 days prior to transplantation of CBA skin. T23 or RmCD4.2+CD8.2 or RbATG (second Ab) were then injected beginning with skin grafting and continued twice a week until rejection: *p* values of statistical significance: 1 *vs.* 2 and 3 = not significant; 4 *vs.* 5 = not significant; 6 *vs.* 7 = not significant; 4 *vs.* 6 = not significant; 5 *vs.* 7 = not significant; 1 *vs.* 5 = 0.022; 3 *vs.* 7 = 0.0064. (B) T23 was preinjected on day -3 followed by RbATG or RmCD4.2+CD8.2 twice weekly for 4 weeks. After an interval of another 4 weeks, mice were boosted as indicated and tolerance to the second Ab was tested by ELISA 1 week after the last booster injection.

Similarly, as in the MmT1/T23 and MmT5/T23 combinations (Sect. 3.1), we were interested to know whether the hyporesponsiveness to RbATG and rat CD4.2+CD8.2 meets the hallmark of tolerance, *i.e.* suppression of anti-second Ab response after rechallenge injections some weeks after discontinuation of treatment. We pre-injected C57BL/6 mice with 400 µg T23 on day -3 followed by 200 µg rabbit ATG or RmCD4.2+8.2 twice weekly for 4 weeks. After 4 weeks without treatment, the mice were boosted with three injections of 20 µg of the second Ab. As shown in Fig. 2B, poor anti-RbATG responses (2.4 ± 0.84 µg/ml) were detected in mice treated with the T23/RbATG combination and rechallenged with RbATG after a 4-week interval. In contrast, control mice injected with RbATG without pre-injection produced a clear anti-RbATG response after rechallenge (112.5 ± 60.4 µg/ml). Comparable results were obtained using the T23/RmCD4.2+CD8.2 combination (Fig. 2B).

3.3 Identification of the T cell subpopulation responsible for the anti-Ig response

In the described experiments, we used pan-T cell mAb in the pre-injection step. To determine the T cell phenotype of the subset, the depletion of which is critical for the observed suppressed responses to the further injections of second Ab, we used the following combinations of first and second Ab: RmCD4.2/MmT1, RmCD8.2/MmT1,

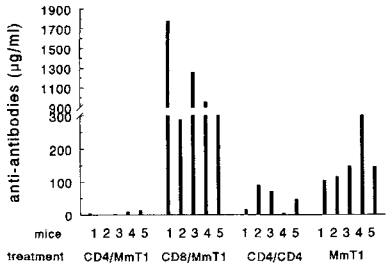

Figure 3. Preinjection of anti-CD4, but not anti-CD8 mAb, suppresses anti-Ab response to booster injections of species-different second Ab. Groups of five C57BL/6 mice were pre-injected with RmCD4.2 or RmCD8.2 (rat IgG2b) on day -3. MmT1 or RmCD4.2 were injected on days 0, 14 and 21, and anti-Ab were determined by ELISA 10 days later.

RmCD4.2/RmCD4.2 and MmT1 alone. Pre-injection of RmCD4.2 inhibits the anti-Ab response to MmT1 (Fig. 3) as effectively as shown for the RmCD4.2+RmCD8.2/MmT1 combination [6]. In contrast, the anti-MmT1 response in mice pre-injected with RmCD8.2 was even stronger than in groups treated with MmT1 without the pre-injection step. As shown, RmCD4.2 was not able to inhibit anti-Ab responses to itself (Fig. 3). It is of note that both RmCD4.2 and RmCD8.2 have comparable high capacities to deplete T cells in C57BL/6 mice (lymph nodes: 3.2 % for $CD3^+CD4^+$ cells 3 days after injection of RmCD4.2, 0.3 % for $CD3^+CD8^+$ cells 3 days after injection of RmCD8.2; control values of untreated controls: 30.8 % for $CD3^+CD4^+$ and 25.2 % for $CD3^+CD8^+$ cells).

3.4 Suppression of anti-Ab to bispecific Ab

Immunogenicity of bispecific Ab has not been studied so far. After generation of rat/mouse quadromas BiB and G2, we examined the immunogenicity of the allo-versus xenogeneic parts of these bispecific mAb and in the applicability of our tolerogenic protocol to inhibit anti-Ab responses to both species components. C57BL/6 mice were immunized with 100 µg G2 or 50 µg BiB i.p. on days 0, 14, 21. In addition, other groups were pre-injected on day -3 with T23 in order to induce hyporesponsiveness. As shown in Fig. 4, injections of BiB induced strong anti-Ab to rat (titer between 1/400 and 1/8000) and moderate to mouse (1/96 and 1/300) Ig. Pre-injection of Fc region-mismatched T23 prevented anti-Ab to BiB (Fig. 4). Injections of G2 resulted in anti-rat Ab titers ranging between 1/768 and

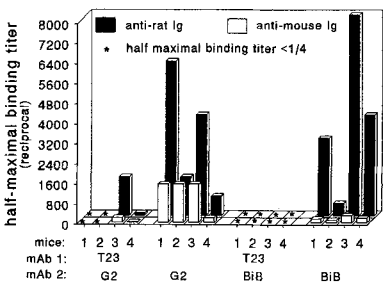

Figure 4. Xenogeneic T23 mAb suppresses anti-Ab response to bispecific Ab. C57BL/6 mice were pre-injected or not with chimeric T23 on day -3 followed by bispecific Ab BiB or G2 on days 0, 14 and 21. Anti-Ab titer was determined by ELISA.

1/6144, while anti-mouse Ig ranged between 1/192 and 1/1536. Similarly, pre-injection of T23 prevented anti-G2 Ab in two of four mice (Fig. 4).

4 Discussion

A major finding in the present study is that Fc region-mismatch between pre-injected first and multiply-injected second T cell-binding Ab is the minimal requirement for prevention of anti-second Ab responses. This difference may be a xenogeneic (mouse/rat, rat/rabbit) or discordant xenogeneic (mouse/human, human/rat, human/rabbit) Fc region mismatch. Generation of recombinant anti-Thy-1.2 mAb T23 with the huIgG1 Fc region and the idiotype of murine anti-Thy-1.2 MmT1 allowed us to relate the immunosuppressive element of Ab mismatch to its C_H region by demonstrating that identity (MmT1/T23) or dissimilarity (MmT5/T23) of idiotypes between first and second T cell depleting Ab was irrelevant for the induction of the immunosuppressive effect (Fig. 1). Tolerance to T23 was found to persist 230 days after graft rejection and termination of Fc region-mismatched Ab treatment, at a time when T cell counts had long since recovered. This tolerance was specific to the isotype of the second Ab, in the case of T23, to the huIgG1 isotype. Thus, upon rechallenge, the T23-tolerant mice produced anti-Ab not only to third-party rat anti-Thy-1 (RmT1), but also to the huIgG3 (T212C8) isotype (Fig. 1C). Interestingly, despite considerable prolongation of 50% graft survival (80–150 days; controls without preinjection of first Ab, 22 days), ultimate rejection occurred during second Ab treatment in mice which showed >90% T lymphocytopenia and no evidence of anti-Ab. Whether down-regulation of T cell Ag in mice under chronic Ab treatment can explain this phenomenon is under study. Clearly, specific tolerance to anti-T cell Ig survived graft rejection in our C57BL/6 mice, a strain where peripheral skin graft tolerance has yet to be shown. Further analysis of this remarkable evolution of specific anti-T cell Ig tolerance underscores the importance of the initial Th cell depletion, which was over 90% by the first anti-pan-T cell or anti-CD4 Ab. Generation of new primary anti-Ab responses against T cell-dependent immunogens (*i.e.* the Fc-mismatched second Ab) should therefore be strongly impaired in mice lacking a sufficient number of reactive Th cell clones necessary for cognate interactions with B cells [15] and for the production of B cell growth and differentiation factors. The continued depletion of Th cells by the second anti-T cell Ab would be likely to result in the long-term suppression of B cell responses during the period of repeated booster injections. As such, it appears that the repeated immunologic challenge of the second Ab during the prolonged absence of Th signals and factors results in specific B or Th cell anergy or clonal deletions. Indeed, we find Ab tolerance to require about 4 weeks of second Ab treatment (Fig. 2B).

Ab treatment with quadroma-derived bispecific Ab has generated increased interest, based on the successful suppression of B cell lymphomas in mice [16–18]. Anti-Ab responses following treatment with bispecific Ab have to date not been reported. To overcome technical difficulties in the purification of sufficient amounts of bispecific mAb for studies *in vivo*, we devised a one-step purification to real bispecificity of reagents which were derived from the fusion of a rat with a mouse hybridoma [11]. We found anti-Ab to the distinct xeno- and allogeneic species components following injections of the bispecific rat/mouse Ab G2 or BiB, which could be prevented by pre-injection of Fc region-mismatched first Ab (Fig. 4). Furthermore, Fc region-mismatched first Ab suppressed anti-Ab to the multiple immunogenic epitopes of polyclonal RbATG (Fig. 2). These experiments illustrate that isotype-specific tolerance induced to second anti-T cell mAb can – at least in the case of polyclonal ATG – be broadened to species-specific tolerance of an as-yet undefined number of idio- and isotypes. Graft survival was at least as much improved as with mAb (Fig. 2).

In summary, we describe a tolerance-inducing approach to suppress anti-Ab to discordant (human/mouse) xenogeneic, bispecific, or polyclonal T cell-binding Ab. It exploits a mismatch between Fc regions of T cell-depleting first and second Ab to induce tolerance to the latter.

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5 References

1 Jaffers, G. J., Fuller, T. C., Cosimi, A. B., Russell, P. S., Winn, H. J. and Colvin, R. B., *Transplantation* 1986. *41:* 572.
2 Jaffers, G. J., Colvin, R. B., Cosimi, A. B., Giorgi, J. V., Goldstein, G., Fuller, T. C., Kurnick, J. T., Lillehei, C. and Russell, P. S., *Transplant. Proc.* 1983. *15, 1:* 646.
3 Miller, R. A., Oseroff, A. R., Stratte, P. T. and Levy, R., *Blood* 1983. *62:* 988.
4 Chatenoud, L., Jonker, M., Villemain, F., Goldstein, G. and Bach, J. F., *Science* 1986. *232:* 1406.
5 Isaacs, J. D., Watts, R. D., Hazleman, B. L., Hale, G., Keogan, M. T., Cobbold, S. P. and Waldmann, H., *Lancet* 1992. *340:* 748.
6 Mysliwietz, J., Thierfelder, S., Mocikat, R. and Kremmer, E., *Eur. J. Immunol.* 1994. *24:* 2323.
7 Thierfelder, S., Kummer, U., Schuh, R. and Mysliwietz, J., *Blood* 1986. *68:* 818.
8 Reinecke, K., Mysliwietz, J. and Thierfelder, S., *Transplantation* 1994. *57:* 458.
9 Kremmer, E., Mysliwietz, J., Lederer, R. and Thierfelder, S., *Eur. J. Immunol.* 1993. *23:* 1017.
10 Miescher, G. C., Schreyer, M. and MacDonald, H. R., *Immunol. Lett.* 1989. *23:* 113.
11 Lindhofer, H., Mocikat, R., Steipe, B. and Thierfelder, S., *J. Immunol.* 1995, in press.
12 Mysliwietz, J., Thierfelder, S., Hoffmann-Fezer, G. and Kummer, U., *Transplantation* 1990. *49:* 749.
13 Clark, M. R., Gilliland, L. and Waldmann, H., in Waldmann, H. (Ed.), *Monoclonal Antibody Therapy. Prog. Allergy,* Karger, Basel 1988, p. 31.
14 Kardinal, C., Hooijberg, E., Lang, P., Zeidler, R. and Mocikat, R., *Eur. J. Immunol.* 1995. *25:* 792.
15 Mitchison, N. A., *Eur. J. Immunol.* 1971. *1:* 18.
16 Demanet, C., Brissinck, J., Van Mechelen, M., Leo, O. and Thielemans, K., *J. Immunol.* 1991. *147:* 1091.
17 Brissinck, J., Demanet, C., Moser, M., Leo, O. and Thielemans, K., *J. Immunol.* 1991. *147:* 4019.
18 Weiner, G. J. and Hillstrom, J. R., *J. Immunol.* 1991. *147:* 4035.