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A MOUSE MODEL FOR THE PRECLINICAL EVALUATION OF IMMUNOSUPPRESSIVE EFFECTOR FUNCTIONS OF HUMAN ISOTYPES

THE HUMAN IGG1 ISOTYPE IS SUPERIOR TO IGG3

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Antibodies against T cells are widely used as immunosuppressive agents in clinical therapy. As effector functions of chimeric or humanized anti-T cell antibodies cannot be predicted in vitro, we compared T cell-depleting effects of human isotypes in vivo with their immunosuppressive consequences in a mouse BMT model. This system is based on chimeric antibodies with a mouse pan T cell specificity and human constant regions. To secure optimal immunosuppression, the specificity for Thy-1.2—one of the best-characterized T cell antigens—was selected, as Thy-1.2-specific antibodies prevent graft-versus-host disease in fully mismatched mice. Chimeric mouse anti-Thy-1.2 antibody with the human IgG1 Fc part was found to be equally effective in preventing graft-versus-host disease mortality as the highly protective anti-Thy-1.2 mouse IgG2a isotype, while human IgG3 was far less effective. This was not predictable by measuring the degree of T cell depletion in peripheral blood. T cell depletion in lymph nodes, however, exactly reflected the results obtained in the BMT system. In addition, this system offers the advantage of assessing the influence of reduced antigen density by using heterozygous Thy-1.2 mice.

The use of mAb for the specific elimination of cells in clinical therapy has attracted much interest (reviewed in *1*). To minimize the induction of neutralizing anti-antibodies (*2*) and to trigger human effector mechanisms more efficiently, chimeric or humanized mAb have been created (reviewed in *3*). When designing agents for specific therapeutic indications, it is important to know which human isotypes provide the desired effector functions in humans. In the hope of predicting such effector functions in vitro, phenomena such as C-dependent cytotoxicity (CDC),* antibody-dependent cell-mediated cytotoxicity (ADCC), or C1q uptake were measured. For these parameters hierarchies were established in vitro for several mouse and rat isotypes (e.g., *4*, *5*). From in vitro studies it is not clear, however, which mechanisms are utilized for mAb-induced cell elimination in vivo. For example, in a recent study, cell depletion in vivo did not correlate with either CDC or ADCC in vitro and ADCC with murine effectors gave variable results depending upon the effector system (*6*). The outcome of in vitro C lysis experiments turned out to be influenced by the C source (*7*). These examples underscore the limitations posed in the interpretation of in vitro data.

The first rodent/human chimeric or humanized mAb used in clinical studies were a T cell-depleting mAb directed against the CAMPATH antigen (*8–10*) and a mAb with specificity for an antigen on gastrointestinal cancer cells (*11*). For both, the human IgG1 isotype was selected on the basis of in vitro studies in which IgG1 displayed the best CDC and ADCC results, followed by IgG3 (*12–15*). Surprisingly, the C1q uptake by IgG1 was, however, less pronounced than by IgG3 (*15*).

Given the uncertainties arising from in vitro investigations, it is necessary to compare the latter two human isotypes with regard to their cell-depleting consequences in vivo. As such experiments in man are not possible, we set out to establish a mouse model for testing the immunosuppressive efficiency of a T cell-depleting mAb with human isotypes. We selected the mouse mAb MmT1 (*16*), which is specific for the murine pan T cell Ag Thy-1.2. It achieves efficient depletion of both CD4 and CD8 cells and suppresses the graft-versus-host reaction after BMT even in fully histoincompatible mice. The immunosuppressive efficacy of MmT1 is correlated with the high Ag density on the target cell (*17*) and to the C1q uptake on mAb-coated cells (*18*, *19*). As there are cross-reactions between Fc-dependent effector mechanisms of man and mouse (*20*, *21*), combining the anti-mouse-Thy-1.2 specificity with human Fc regions provides a prognostic model for the preclinical evaluation of T cell-depleting effector functions of human isotypes.

While our work was in progress, a similar system was described using rat/human chimeric anti-mouse-CD8 mAb (*21*). This study also confirmed the assumption that Fc-dependent effector mechanisms are exchangeable between mouse and man. Here, no difference was observed between the degree of T cell depletion in peripheral blood induced by human IgG1 and IgG3. mAb against the $CD8^+$ subset, however, do not sufficiently suppress graft-versus-host disease (GVHD) in fully mismatched mice (*22*). We have extended this work in that we have used an anti-pan-T cell mAb. By measurement of T cell depletion in lymphoid organs and by assessing immunosuppression in a GVHD model in mice, we detected a differential immunosuppressive potential of human IgG1 and IgG3.

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* Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, C-dependent cytotoxicity; GVHD, graft-versus-host disease; PCR, polymerase chain reaction.

MATERIALS AND METHODS

Cell lines. MmT1 is a mouse hybridoma (γ2a/κ) with specificity for the Thy-1.2 Ag (*16*). P388D_1 is a mouse macrophage-like cell line (*23*) and Sp2/0 is a mouse myeloma cell line (*24*). Cells were cultured in RPMI 1640 medium supplemented with 2 mM glutamine and 5% FCS (Gibco BRL, Eggenstein, Germany). For large-scale production of chimeric mAb, low protein medium (Gibco BRL) was used.

Construction of expression vectors. Polymerase chain reaction (PCR) amplification of the V_H and V_κ gene from MmT1 has been described previously (*25*). After digestion with *PstI/BstEII* (H chain) or *SacI/BglII* (κ-chain), the PCR products were cloned into pUC18 or pSP70, respectively, in which the *HindIII/BamHI* fragments of the constructs M13-VHPCR1 or M13-VKPCR1 (gifts from Dr. G. Winter, Cambridge, UK) had been inserted (*26*). The functional V genes were identified by sequencing. For the construction of the IgG1 H chain expression vector, the 2.9-kb *EcoRI/PvuII* fragment carrying the human IgG1 C gene segments was excised from pUC13 (*27*) and ligated into *EcoRI/BamHI*-digested pSV2 gpt (*28*) whose *BamHI* site had been blunted with Klenow enzyme. The 0.7-kb *EcoRI/XbaI* fragment containing the murine μ-intron enhancer was inserted into the *EcoRI* site upstream of the IgG1 exons. The V_H gene including the Ig promoter was excised from pUC18 with *HindIII/BamHI*, cloned into pGEM7zf (Promega, Madison, WI), excised as an *EcoRI/SacI* fragment, and inserted between the *EcoRI* and *SacI* site 5′ of the IgG1 exons (vector pSVgpt-Eμ2-V_HMmT1-huγ1).

For the construction of pSVgpt-V_HMmT1-huγ3 a 2.9-kb *EcoRI/SphI* fragment including the human IgG3 segments (*29*) was blunt-end-ligated into pSVgpt-Eμ2-V_HMmT1-huγ1 whose C region was excised.

The human C_κ gene segment (*30*) is located on a 1.9-kb *EcoRI/PvuII* fragment which was ligated into *EcoRI/SmaI*-digested pUC18 together with the murine μ-intron enhancer, excised with *EcoRI/BamHI*, and inserted into pSV2neo. The V_κ gene, which is included in a *HindIII/BamHI* fragment in pSP70, was inserted into pSP72 and then transferred as an *XhoI/EcoRI* fragment into *SalI/EcoRI* cut pSV2neo which contained the C_κ segment and the μ-enhancer. This plasmid was named pSVneo-V_κMmT1-E-C_κ.

Transfection protocol. In a standard electroporation experiment, the H chain and κ-chain vectors were linearized with *PvuI*, mixed, precipitated with isopropanol, and resolved in deionized water. Three to 5×10^6 exponentially growing Sp2/0 cells were washed with PBS, suspended in 700 μl PBS, and mixed with 3–5 μg of each vector. After 10 min on ice, the cells were exposed to a single pulse of 200 V, 960 μF, in a Bio-Rad (München, Germany) electroporation apparatus. The cells were kept on ice for an additional 10 min and then plated on 96-well dishes at a density of 10^4 cells/well. On day 3, we began selection with 0.7 mg/ml G418 (Gibco BRL), and on day 5 we began additional selection with increasing amounts of mycophenolic acid.

ELISA. To screen the stable transfectants, ELISA plates were coated with goat anti-human-IgGFc (Dianova, Hamburg, Germany) and blocked with 1% milk powder in PBS. The plates were incubated with culture supernatants and then with goat anti-human-IgGFab coupled with horseradish peroxidase. The color reaction was initiated with *o*-phenylenediamine (Sigma, München, Germany).

mAb purification. mAb were purified to homogeneity by fast performance liquid chromatography on a protein G sepharose column (Pharmacia, Freiburg, Germany) according to the manufacturer's protocol and extensively dialyzed against PBS. The mAb concentration was determined according to Ref. *31*.

FACS analyses. Flow cytometry, binding studies, and competitive inhibition assays were performed on a FACScan (Becton Dickinson, Heidelberg, Germany) using quantitative fluorescein microbead standards (Becton Dickinson) as described (*32*).

C1q binding assay. The C1q uptake was monitored by a cell ELISA using lymph node cells from C57BL/6 mice that were incubated with 10–20 μg/ml mAb and then with human or mouse C or purified human C1q (Calbiochem, La Jolla, CA) at various dilutions. C1q was detected by peroxidase-conjugated goat anti-human-C1q antibody (Dianova) or by 7H8, a monoclonal rat anti-mouse-C1q antibody (*19*).

C-mediated cytotoxicity. Lymph node cells from C57BL/6 mice were purified on a Percoll gradient. They were coated with mAb at a concentration of 20 μg/ml for 30 min at room temperature and then incubated with diluted mouse or human serum for 45 min at 37°C. Human serum has been preadsorbed with spleen and lymph node cells from C57BL/6 mice for 45 min on ice. The percentage of lysed cells was determined in the FACS.

Depletion experiments. C57BL/6 or C57BL/6 (Thy-1.1×Thy-1.2) F1 mice were injected intraperitoneally with 180–250 μg of mAb. After 4 days, the animals were killed. Lymph node, spleen, and peripheral blood cells were isolated, double-labeled with anti-CD3/anti-CD4, anti-CD3/anti-CD8 or anti-human IgGFc/anti-mouse μ, and quantitated in the FACS. The FITC- and phycoerythrin-conjugated polyclonal antibody were purchased from Dianova.

For immunohistochemical analysis, cryostat sections (5 μm) from spleen and lymph nodes were air-dried, fixed in acetone for 10 min, and incubated with the following antibody for 60 min: peroxidase-labeled anti-human-κ as single incubation or biotinylated rat anti-mouse-CD3, -CD4, -CD8 or rat normal serum which were followed by peroxidase-labeled avidin. After washing in Tris-buffered saline, peroxidase activity was revealed with 3-amino-9-ethylcarbazole. The tissue sections were counterstained with hematoxylin.

BMT experiments. BMT was performed as described elsewhere (*33*). In brief, CBA (H-2^k) mice were exposed to gamma irradiation of 850 cGy using a ^{137}Cs source and, after 4 hr, injected intraperitoneally with 500 μg mAb in PBS. After 24 hr, 5×10^7 spleen and 2×10^7 bone marrow cells were injected into the tail vein. For donor mice, we used C57BL/6 (H-2^b) animals or C57BL/6 (Thy-1.1×Thy-1.2) F1 hybrids.

RESULTS

Cloning of the V genes from MmT1 and expression of chimeric mAb. The functionally rearranged V gene segments of the mouse anti-Thy-1.2 hybridoma MmT1 were isolated by PCR as described previously (*25*) and identified by sequencing. The complete nucleotide and deduced amino acid sequences are depicted in Figure 1. The H chain was assigned to subgroup IB, although the proline residue at position 14, which is highly conserved in H chains of this subgroup, is substituted by a threonine. The κ-chain belongs to subgroup IV, although there is a glycine at position 61 instead of an alanine. These substitutions were confirmed by independent amplifications.

The V genes were cloned into expression vectors that contained an Ig H chain promoter, the murine μ-intron enhancer, and either the human IgG1 or IgG3 C exons or the C_κ gene segment. The H and the κ-chain constructs were expressed after cotransfection into Sp2/0 cells. The resulting chimeric anti-Thy-1.2 mAb were named Ko1 (human IgG1 isotype) and γ3–7 (human IgG3 isotype).

Characterization of the chimeric anti-Thy-1.2 mAb. For all experiments described below, we used mAb preparations that were purified to homogeneity by fast performance liquid chromatography on protein G Sepharose. The integrity of the recombinant proteins was demonstrated by reducing and nonreducing SDS-PAGE and Western blots which confirmed the correct sizes of the IgG1 H chain (55 kDa), the IgG3 H chain (60 kDa), the κ-chain (about 30 kDa), and the nonreduced mAb (data not shown). The binding specificity and avidity were determined in inhibition assays. Binding of FITC-labeled MmT1 on C57BL/6 lymph node cells was competed for by serially diluted MmT1, Ko1, or γ3–7 and quan-

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CAG GTC CAA CTG CAG CAG TCT GGA CCT GGC CTA GTG CAG ACC TCA CAG AGC CTG   54
 Q  V  Q  L  Q  Q  S  G  P  G  L  V  Q  T  S  Q  S  L                   18

TCC ATC ACC TGC ACA GTC TCT GGT TTC TCA TTA ACT AAC TTT GGT ATA CAC TGG  108
 S  I  T  C  T  V  S  G  F  S  L  T  N  F  G  I  H  W                   36

ATT CGC CAG TCT CCA GGA AAG GGT CTG GAG TGG CTG GCA GTG ATA TGG AGT GGT  162
 I  R  Q  S  P  G  K  G  L  E  W  L  A  V  I  W  S  G                   54

GGA AGC ACA GAC TAT AAT GCA GCT TTC ATA TCC AGA CTG ACC ATC AGC AAG GAC  216
 G  S  T  D  Y  N  A  A  F  I  S  R  L  T  I  S  K  D                   72

AAC TCC AAG AGC CAA GTT TTC TTT AAA ATG AAC AGT CTG CAA GCT GAT GAC ACA  270
 N  S  K  S  Q  V  F  F  K  M  N  S  L  Q  A  D  D  T                   90

GCC ATA TAC TAC TGT GCC AGA GCC ATT CAT TAC TAC GGT AGT GGC TAC CGG TAC  324
 A  I  Y  Y  C  A  R  A  I  H  Y  Y  G  S  G  Y  R  Y                  108

TTC GAT GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA                  378
 F  D  V  W  G  Q  G  T  T  V  T  V  S  S                               126
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A

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GAG CTC GTG CTC ACC CAG TCT CCA GCA ATC ATG TCT CGA TCT CCA GGG GAG AAG   54
 E  L  V  L  T  Q  S  P  A  I  M  S  R  S  P  G  E  K                   18

GTC ACC ATG ACC TGC AGG GCC AGC TCA AGT GTA AGT TCC AGT TAC TTG CAC TGG  108
 V  T  M  T  C  R  A  S  S  S  V  S  S  S  Y  L  H  W                   36

TAC CAG CAG AAG CCA GGA TCT TCC CCC CAA CTC TGG ATT TAT AGC ACA TCC AAC  162
 Y  Q  Q  K  P  G  S  S  P  Q  L  W  I  Y  S  T  S  N                   54

CTG GCT TCA GGA GTC CCA GGT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC  216
 L  A  S  G  V  P  G  R  F  S  G  S  G  S  G  T  S  Y                   72

TCT CTC ACA ATC AGC AGT GTG GAG GCT GAG GAT GCT GCC ACT TAT TAC TGC CAG  270
 S  L  T  I  S  S  V  E  A  E  D  A  A  T  Y  Y  C  Q                   90

CAG TAT GAT AGT TCC CCG TAT ACG TTC GGA TCG GGG ACC AAG CTG GAG ATC AAA  324
 Q  Y  D  S  S  P  Y  T  F  G  S  G  T  K  L  E  I  K                  108
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B

FIGURE 1. Nucleotide and deduced amino acid sequences of the (A) H chain and (B) κ-chain V gene segments of the mAb MmT1. The complementarity determining regions are underlined; PCR primers are printed in italics. The sequences are available at the EMBL/GenBank library under the accession numbers X58292 and X58293.

titated in the FACS. The chimeric mAb exhibited essentially the same binding avidity as MmT1 (not shown).

C1q fixation by chimeric mAb. The C1q uptake of the chimeric anti-Thy-1.2 mAb was measured in an ELISA using lymph node cells that were coated with mAb in saturating concentrations and then incubated with decreasing amounts of mouse or human serum or purified human C1q. The bound C1q was detected by peroxidase-labeled anti-human-C1q or by a monoclonal anti-mouse-C1q antibody, respectively. For comparison, we included the parent mouse γ2a mAb MmT1 in our analyses.

As shown in Figure 2A, the mouse IgG2a, the human IgG1, and the human IgG3 isotype display a comparable C1q affinity when mouse serum is used as C source. With human serum, a slightly more efficient C1q fixation was observed for MmT1 than for the human isotypes, but the behavior of Ko1 and γ3–7 was identical (Fig. 2B). When purified human C1q was used, there was, however, a marked difference between Ko1 and γ3–7 in as much as human IgG1 was binding C1q much less efficiently than IgG3 or mouse IgG2a (Fig. 2C). We obtained the same results when mAb were serially diluted and C1q was added in saturation.

C-mediated cytotoxicity. For C-dependent cell lysis experiments, we used human and mouse serum as C source in order to provide a better estimation of the in vivo situation. Lymph node cells were lysed in the presence of saturating amounts of mAb and various dilutions of serum. Cell survival was determined in the FACS. The outcome of a typical experiment is shown in Figure 3. There was no difference in CDC between Ko1 and γ3–7 regardless of the C source. In low dilu-

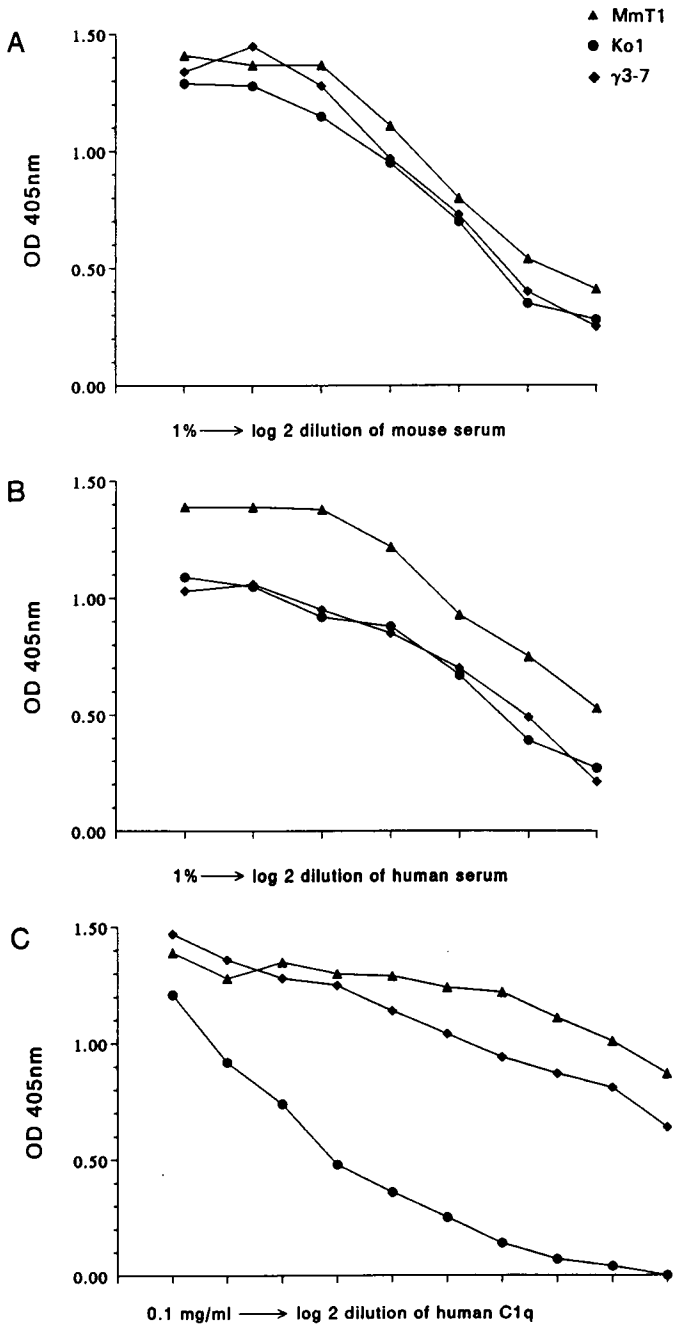


FIGURE 2. Typical result of an experiment measuring the C1q uptake by MmT1, Ko1, and γ3–7 with mouse (A) and human (B) complement and purified human C1q (C). For details see text.

tions of human serum, cells are lysed also in the absence of mAb, because human serum is toxic per se. The original mouse IgG2a mAb MmT1 exerted the same cytotoxic effect as the two human isotypes (not shown).

FcR binding. The relative FcR binding affinities of the chimeric mAb were determined using the murine macrophage cell line $P388D_1$ (*23*), which turned out not to express the Thy-1.2 Ag (not shown) and displays high and low affinity FcR. FcR binding of FITC-labeled MmT1 was inhibited by decreasing concentrations of MmT1, Ko1, or γ3–7. γ3–7 and MmT1 displayed identical affinities for FcR, whereas Ko1 reproducibly bound with a markedly lower affinity (Fig. 4).

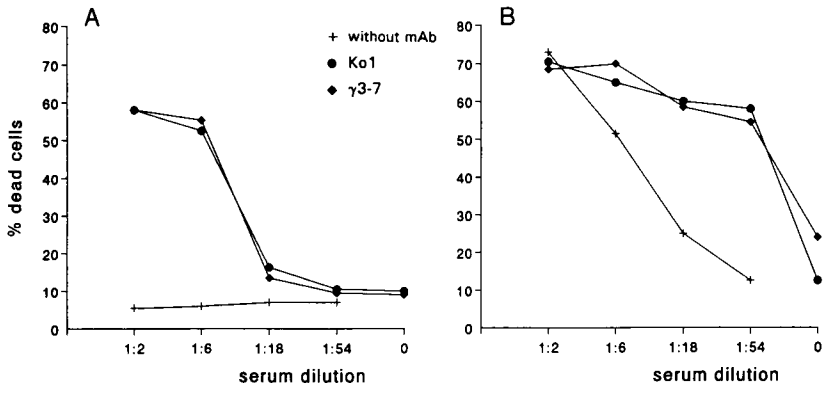


FIGURE 3. C-mediated lysis of lymph node cells with mouse (A) or human (B) serum as C source. For details see text.

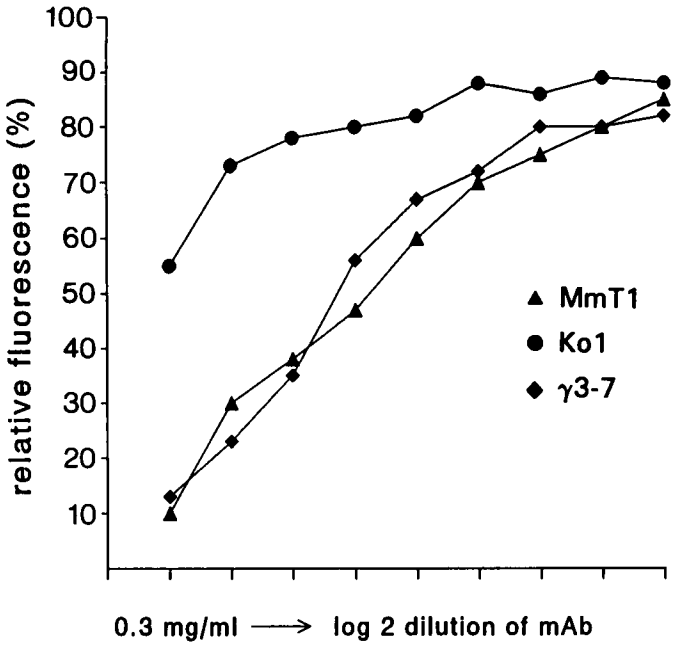


FIGURE 4. Inhibition of binding of FITC-labeled MmT1 to FcR of $P388D_1$ cells by decreasing amounts of unlabeled MmT1, Ko1, and γ3–7. The mean fluorescence intensities are given as percentage of the control value without unlabeled mAb.

T cell depletion in mice. Keeping the ambiguities of in vitro results in mind, we then compared the T cell-depleting effector functions of the human isotypes in vivo. From 180 to 250 μg of mAb were delivered to C57BL/6 mice in a single dose. After 4 days, the percentage of cells staining for CD3/CD4 and CD3/CD8 in peripheral blood, spleen, and lymph nodes was determined in the FACS. The results are summarized in Table 1. In all tissues analyzed, no significant difference was found between the T cell-depleting potential of Ko1 and that of the parent mouse mAb MmT1. This indicates that mouse effector functions can actually be recruited by human Fc regions. The IgG3 isotype, however, turned out to be less effective than IgG1. Whereas the difference between Ko1 and γ3–7 was not clearly detectable in peripheral blood and in the spleen, it was very pronounced in lymph nodes. The residual cells which could be detected after depletion with Ko1 or γ3–7 were coated with the chimeric mAb. While in blood residual T cells displayed practically equal staining for human IgGFc regardless of the isotype used for depletion, the mean fluorescence intensity (i.e., the coating density) of cells from lymph nodes depleted with γ3–7 was only 45.1% as compared with that of lymph node cells from Ko1-depleted mice.

Analogous results were obtained by immunohistochemical analyses which revealed a differential effect of Ko1 and γ3–7 not only in lymph nodes, but also in the spleen. Figure 5 shows representative spleen sections from mice depleted with Ko1 (Fig. 5b) or γ3–7 (Fig. 5c) in comparison to one from an untreated mouse (Fig. 5a). Control mice treated with MmT1 yield the same picture as mice depleted with Ko1 (not shown).

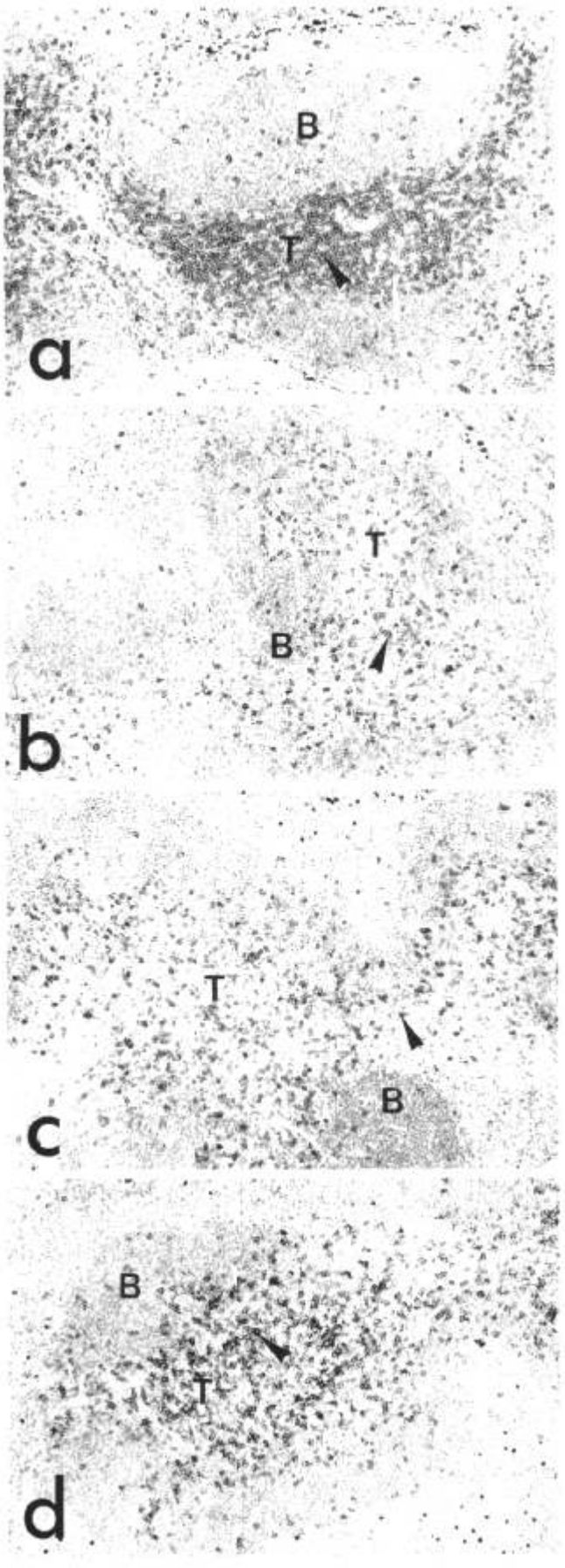
To confirm these findings in a system which should be more sensitive for evaluating T cell-depleting efficiencies of mAb, we repeated the described experiments in C57BL/6 (Thy-1.1×Thy-1.2) F1 mice which express the Thy-1.2 Ag at lower density (J. Mysliwietz, personal communication, 1992). FACS analyses (not shown) and immunohistology revealed the same hierarchy of Ko1 and γ3–7. As expected, T cell depletion by both isotypes was less pronounced than in the parent Thy-1.2 mice (Fig. 5d).

Half-lives of chimeric mAb in mice. To rule out the possibility that the different behavior of Ko1 and γ3–7 was due to different serum half-lives, C57BL/6 Thy-1.1 mice were injected with 100 μg of Ko1 or γ3–7 via the tail vein and bled at various times up to 29 days p.i. The serum concentrations were determined by ELISA. Both chimeric mAb were elimi-

TABLE 1. T cell depletion by MmT1 and chimeric anti-Thy-1.2 mAb in C57BL/6 mice[a]

	Peripheral blood		Spleen		Lymph nodes	
	CD3/CD4	CD3/CD8	CD3/CD4	CD3/CD8	CD3/CD4	CD3/CD8
Untreated	18.0	12.0	15.0	8.0	30.0	25.0
MmT1	0.2	0.2	2.4	0.6	5.5	3.4
Ko1	0.6	0.3	3.3	0.8	9.1	2.8
γ3–7	1.8	0.7	4.7	1.5	19.7	6.4

[a] Percentage of $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cells 4 days after a single injection of mAb. Each value represents the mean from 4 mice.



nated from the circulation with identical biphasic kinetics (not shown). The rates of clearance were indistinguishable from those reported for a mouse IgG2b mAb in C57BL/6 mice (*34*).

A GVHD model for the assessment of immunosuppression by human isotypes. A BMT model using fully histoincompatible mice provides a sensitive system for evaluating the immunosuppressive efficacy of an anti-T cell mAb. Requiring a single injection and relying on the measurement of mortality, this system is very convenient and objective. Therefore, we tested our chimeric anti-Thy-1.2 mAb for their capability to suppress the graft-versus-host reaction after BMT using C57BL/6 (H-2^b) mice as donors and CBA (H-2^k) mice as recipients. MmT1 was able to prevent GVHD mortality in these animals, which are fully mismatched for MHC and minor antigens. The recipients were irradiated and received a single-dose injection 20 hr before BMT. The results are depicted in Figure 6. Again, the human IgG1 isotype was found to be much more effective than IgG3. Ko1 and the highly protective MmT1 turned out to equally induce long-lasting immunosuppression, whereas γ3–7 was not capable of preventing GVHD mortality.

To assess the immunosuppression by Ko1 in mice that display the target Ag at a density comparable to that of CD8, e.g., Ko1 was also tested in BMT experiments using C57BL/6 (Thy-1.1×Thy-1.2) F1 animals as donors. In this system, only an intermediate prolongation of survival could be achieved, as it was also observed for MmT1.

DISCUSSION

A specific tool for the induction of a long-lasting immunosuppression in clinical therapy is the depletion of all T cell subsets with mAb. The in vivo test system for T cell-depleting effector functions of human isotypes described in this article is based on coating of mouse T cells with mAb to the pan T cell Ag Thy-1.2, whose high density allows efficient immunosuppression even in fully histoincompatible mice. Taking advantage of the fact that (Thy-1.1×Thy-1.2) hybrids express Thy-1.2 at reduced density, our system allows evaluation of GVHD suppression in fully mismatched mice also under conditions of mAb coating comparable to those obtained by anti-CD8 or -CD4.

The hierarchy found for C1q uptake (Fig. 2) is in agreement with that established for CDC (Fig. 3) as far as mouse serum was used as C source (mouse γ2a = human γ1 = human γ3). Purified human C1q bound less efficiently to human IgG1, so that we have to postulate additional components in the serum necessary for stabilizing C1q binding to this isotype. The results obtained with human serum (mouse γ2a > human γ1 = human γ3 for C1q uptake; mouse γ2a = human γ1 = human γ3 for CDC) are in contrast to other studies which established other rank orders for C1q binding (*15*) and CDC (*12*, *14*, *15*, *35*) with human C. This disparity may be explained by influences of the Ag density and the

FIGURE 5. Immunohistochemical staining of $CD3^+$ T lymphocytes (arrowheads) in spleen white pulp obtained from (a) an untreated C57BL/6 mouse, from C57BL/6 mice depleted with (b) Ko1 or (c) γ3–7, and from (d) a C57BL/6 (Thy-1.1×Thy-1.2) F1 mouse depleted with Ko1. A clear hierarchy in the degree of T cell depletion can be seen in panels b to d. T, periarteriolar T cell zone; B, peripheral B cell area. Magnification ×95.

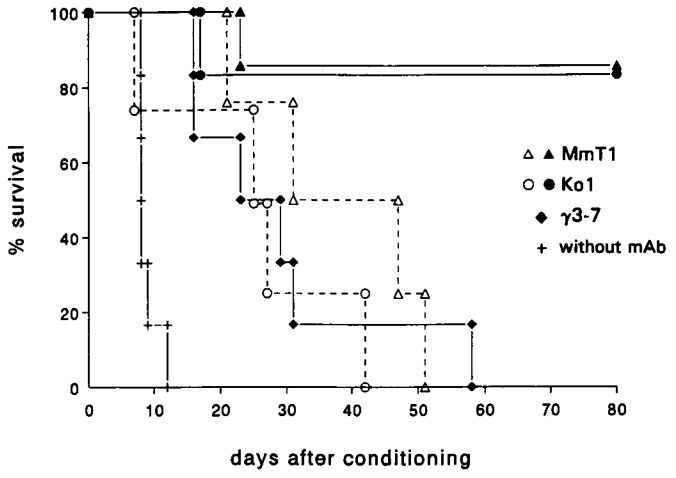


FIGURE 6. Survival of CBA mice after transplantation of spleen and bone marrow cells from C57BL/6 mice (solid lines, closed symbols) or C57BL/6 (Thy-1.1×Thy-1.2) F1 hybrids (broken lines, open symbols). The recipients were conditioned with 850 cGy and 500 μg of the indicated mAb. For each experiment, there were 6 animals in each group (or 4 in the case of the F1 hybrids). For each mAb, 1 experiment out of 2 is shown.

quality of Ag binding, as in these reports specificities for the Tac protein (*35*), a tumor-associated Ag (*12*), the CAMPATH Ag (*14*), or NP-coated erythrocytes (*15*) were investigated. Also, the CDC experiments performed by Isaacs et al. (*21*), who found a more effective lysis by human IgG3 than by IgG1, are not comparable to our results, because these authors used rabbit C exclusively and because the C source influences the results of in vitro studies (*7*). The hierarchy for binding of the human isotypes to murine FcR (human γ3 > human γ1; Fig. 4) was the same as described previously (review in *36*).

When the chimeric anti-Thy-1.2 mAb were tested in vivo, the human IgG1 isotype was shown to be equivalent to the potent C1q-high-affine mouse IgG2a parent mAb. This again emphasizes the possibility of recruiting murine effector functions by human Fc regions and points to the usefulness of our approach as a prognostic model. In contrast, the human IgG3 subclass did not prevent GVHD mortality (Fig. 6). This disparity is accounted for neither by different serum half-lives nor by differences in Ag binding avidity. The difference in GVHD suppression was only reflected by T cell depletion in lymphoid organs, but not in peripheral blood.

The in vivo result neither correlated with C1q fixation nor CDC nor FcR binding. Hence, the differential effect of IgG1 and IgG3 points to additional mechanisms which might only become detectable in vivo, such as differential tissue permeability. In this context, it is interesting to note that the residual T cells in the lymph nodes were less densely coated with chimeric mAb after depletion with γ3–7 than after depletion with Ko1. In the blood, in contrast, both isotypes yielded the same cell coating on the residual T cells after depletion. mAb coating on lymph node cells from an untreated mouse in vitro was also identical. We speculate that IgG3 might penetrate less efficiently into lymph nodes than IgG1. As in other studies (*6*), these results highlight the insufficient capability of in vitro parameters for the prediction of the therapeutic potential of a mAb and emphasize the necessity of in vivo models.

Our results contrast with those reported by Isaacs et al. (*21*), who found identical T cell depletion by human IgG1 and IgG3 in mice. These authors, however, used anti-CD8 chimeric mAb, thereby targeting just the cytotoxic/suppressor subpopulation of the T cell repertoire. The different coating densities of anti-CD8 and anti-Thy-1.2 mAb cannot explain the discrepancy, as Ko1 and γ3–7 exhibited different effects also in mice expressing the Thy-1.2 Ag at a density comparable to that of CD8, i.e., in (Thy-1.1×Thy-1.2) F1 hybrids. A direct comparison of the two studies is further complicated in that Isaacs et al. treated thymectomized mice in 3 divided mAb doses. We decided to use euthymic mice in order to mimic the clinical situation more closely. This should allow a realistic estimation of the depleting potential, taking into account that repopulation from the thymus may impede the T cell-depleting effect of a mAb. To avoid interference by anti-antibodies following multiple injections of mAb, we delivered single-dose injections. Isaacs et al. measured T cell depletion only in peripheral blood. The question remains as to whether T cell clearance in the blood reflects the degree of T cell depletion in other organs and the overall immunosuppression obtained. In another study, we found that pronounced T cell depletion by anti-mouse-CD3 in peripheral blood did not reflect depletion in spleen and lymph nodes (*37*). Also in the present work the difference between IgG1 and IgG3 was not as clearly visible in the peripheral blood as in lymphoid organs. In FACS analyses, only the degree of T cell depletion found in lymph nodes clearly paralleled the differential effect of IgG1 and IgG3 in the BMT experiments. Immunohistological techniques were able to detect this difference in the spleen also.

Taken together, our GVHD suppression model provides a sensitive system for detecting differences of human isotype-related T cell-depleting effector functions in vivo. It measures immunosuppression by mortality, which is an objective endpoint. Finally, by using homo- or heterozygous Thy-1.2 donor cells, its sensitivity can be adapted to the potency of the effector functions of the isotype in question.

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