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# $CD3^+$ T cells in severe combined immunodeficiency (scid) mice

## VI. RESCUE OF SCID-DERIVED, IGM-PRODUCING B CELLS BY TRANSFER OF $CD4^+$ $CD8^-$ T CELLS FROM VARIOUS LYMPHOID ORGANS

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### SUMMARY

Intravenous injection of purified $CD4^+$ $CD8^-$ T cells from thymus, spleen or lymph nodes of adult dm2 donor mice (H-$2^d$, $L^{d-}$, $IgM^a$) into young C.B-17 scid/scid (scid) mice (H-$2^d$, $L^{d+}$, $IgM^b$) partially and selectively reconstituted the splenic $CD4^+$ T-cell compartment of recipient scid mice. This was demonstrated by cytofluorographic analyses, histological examinations, growing donor-derived $CD4^+$ T-cell lines *in vitro* from spleens of transplanted scid mice, and serial passage of $L^{d-}$, $CD3^+CD4^+CD8^-$ T-cell lines through young scid recipients for more than 1 year. Host-derived $IgM^b$ appeared in sera of all $CD4^+$ T-cell-transplanted young scid mice, while none of the sex- and age-matched, non-transplanted control scid mice was $Ig^+$. B-cell *leakiness* was most efficiently induced by transfer of low numbers of adult $CD4^+$ $CD8^-$ thymocytes: transfer of $10^3$ purified $CD4^+$ $CD8^-$ thymocytes efficiently rescued scid-derived B cells, while transfer of $2 \times 10^7$ non-fractionated thymocytes from the same donor mouse was inefficient. Serum antibodies of scid mice with T-cell-induced B-cell leakiness stained congenic 'double-positive' ($CD4^+$ $CD8^+$) thymocytes and immunoprecipitated protein bands with an apparent MW of 14,000, 28,000, 43,000, 65,000 and 80,000 from $^{125}I$-labelled thymocytes. These data indicate that repopulation of peripheral lymphoid organs with $CD4^+$ T cells is always accompanied by partial co-reconstitution of the B-cell system, and raises the question of the interdependence of these two lymphocyte subsets.


### INTRODUCTION

Competent T and B cells are not detected in C.B-17 (H-$2^d$) mice homozygous for the autosomal recessive mutation 'severe combined immunodeficiency' (scid) on chromosome 16.[1-3] This mutation affects recombinase activity involved in DNA re-arrangement processes of gene segments encoding antigen receptors of lymphocytes.[4,5] Because of defective re-arrangements of immunoglobulin (Ig) and T-cell receptor (TcR) gene segments, functional antigen receptors are not expressed by lymphocytes from scid mice. In this mutant mouse strain the peripheral T- and B-cell compartments are therefore 'empty'.

With increasing age C.B-17 *scid/scid* (scid) mice 'spontaneously' develop T and B cells, i.e. become 'leaky'.[6-14] Leaky scid mice always show oligoclonal development of both T and B cells:[6-11] variable amounts of Ig appear in sera of previously Ig-negative scid mice, and $CD3^+$ T cells are detectable in the spleen and peritoneal cavity. The overall number of antigen receptor-expressing lymphocytes in leaky scid mice is low. 'Spontaneous leakiness' is a somatic event observed in most scid mice more than 1 year of age.[3,6,9]

Oligoclonal development of lymphocytes in scid mice is inducible. The leaky phenotype is observed with increasing frequency in scid mice transferred from a specific pathogen-free (SPF) to a non-SPF environment.[8] This suggests a role of microbial antigens in rescuing and/or expanding leaky lymphocytes. Alternatively, stimulation by autoantigens may drive development of leaky lymphocytes in scid mice. T cells are expanded in scid mice after repeated injections of monoclonal antibodies (mAb) produced by B hybridomas from leaky $Ig^+$ scid mice.[12] B cells of young scid mice are rescued by transplantation of neonatal BALB/c thymocytes[13] or autoreactive BALB/c $CD4^+$ T-cell lines.[14] The latter data suggest a role for T–B interactions in peripheral lymphocyte expansion, which is supported by the observation that T and B cells always co-develop in 'spontaneously' leaky scid mice.

In the present study we analysed the rescue of IgM-producing B cells in young scid mice by intravenous transfer of

Abbreviations: LNC, lymph node cells; SC, spleen cells; scid, severe combined immunodeficiency; THY, thymocytes.

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limiting numbers of purified CD4+ CD8− T cells from central or peripheral lymphoid organs of adult dm2 donor mice. We have previously shown that despite the H-2 class I major histocompatibility barrier in this strain combination, no graft-versus-host disease develops in such transplantation experiments.[15] The described data indicate that low numbers of donor CD4+ T cells engrafted in scid recipients always rescue scid B cells in the process of repopulating the immunodeficient host.

## MATERIALS AND METHODS

*Mice*

C.B-17 *scid/scid* (scid) mice (H-$2^d$, $IgH^b/IgM^b$, $L^{d+}$) and allotype-congenic BALB/c H-$2^{dm2}$ (dm2) mice (H-$2^d$, $IgH^a/IgM^a$, $L^{d-}$) were bred under SPF conditions in the animal colony of the Institute of Microbiology, Ulm University, Ulm, Germany. Breeding pairs of C.B-17 *scid/scid* mice were a generous gift from Dr R. A. Phillipps (Toronto, Canada). Male and female mice were used at the age indicated.

*Cell suspensions*

Spleen cells (SC), thymocytes (THY) or lymph node cells (LNC) were aseptically prepared from at least five adult dm2 donor mice, pooled and freed from red blood cells by brief incubation in 0·84% ammonium chloride. In transplantation experiments lymphoid cell populations were passed through nylon wool columns. Non-adherent cells were labelled for cell sorting. Cell sorter-purified CD4+CD8− cells were injected intravenously into 3–4-week-old, sex-matched scid mice.

*Immunofluorescence staining and cytofluorometric analysis*

The following reagents and mAb were used: FITC-conjugated anti-CD8 (Ly-2) mAb 53-6.72 and PE-conjugated anti-CD4 (L3T4) mAb GK1.5 (Becton-Dickinson, Mountain View, CA; Cat. nos. 1353 and 1447); FITC- or biotin-conjugated anti-CD3 (ε-chain) mAb 145-2C11 (a generous gift of Dr J. Bluestone, Chicago, IL); and PE- or FITC-conjugated streptavidin (Dianova/Jackson Immunoresearch Labs Inc., Hamburg, Germany; Cat. nos. 016-090-084 and 016-100-084). The biotin-conjugated anti-$L^d$ mAb 28-14-8S [American Type Culture Collection (ATCC), Rockville, MD] was used to distinguish donor- and host-derived cells. The mAb 145-2C11 was purified from ascites fluid by ammonium sulphate precipitation and passage through protein G–sepharose columns (Pharmacia, Uppsala, Sweden). All reagents (except PE conjugates) were centrifuged at 100,000 ***g*** for 30 min prior to staining to remove aggregated material (Beckman Airfuge, Palo Alto, CA).

Cells were washed and stained in phosphate-buffered saline (PBS)/0·3% (w/v) bovine serum albumin (BSA). Cells were incubated with mAb (1 μg/$10^6$ cells) for 20 min at 4°, and washed twice. In some experiments, cells were subsequently incubated with a second-step reagent for 20 min at 4°. Non-adherent cells were double-labelled with PE-conjugated anti-CD4 mAb and FITC-conjugated anti-CD8 mAb, and CD4+CD8− cells were separated by cell sorting. Relative fluorescence intensity of individual cells was measured using the Epic V flow cytometer (Coulter Inc., Hialeah, FL). The forward narrow angle light scatter was used as an additional parameter to facilitate exclusion of dead cells and aggregated cell clumps. Cells were sorted under sterile conditions. Sorted cell populations were washed, counted and injected intravenously into scid mice. Cell sorter-purified cell populations were routinely re-analysed for purity. In all experiments described, the purity was >96%. B-cell contamination of cell sorter-purified CD4+ T-cell populations was always below 0·5%. For cytofluorographic marker profile studies, cells were suspended in medium supplemented with 0·2% w/v sodium azide.

*Determination of serum immunoglobulin levels (ELISA)*

Blood was obtained from mice by cardiac puncture. An ELISA was used to quantify serum Ig concentrations. Microtitre wells were coated with purified goat anti-mouse Ig (H- and L-chain) (Sigma Chemical Co., St Louis, MO; Cat. no. M6149) and incubated with titrated concentrations of serum. Bound Ig was revealed by purified polyvalent goat anti-mouse antibodies conjugated to alkaline phosphatase (Sigma; Cat. no. A0162). The reference antigen was purified mouse IgG (Sigma; Cat. no. 15381). The relative amount of bound enzyme conjugate in each well was measured by the extent of p-nitrophenylphosphate hydrolysis (Sigma; Cat. no. N9389) was detected at 405 nm in a Micro-Elisa Reader (Dynatech MR5000, Alexandria, VA). Serium Ig concentrations were calculated from the standard curve. The detection threshold for mouse Ig of this ELISA was 20 ng/ml.

For allotype-specific determinations of serum IgM titres, an ELISA was set up using two different combinations of anti-allotypic mAb. In the first allotype-specific ELISA, microtitre wells were coated with purified anti-mouse $IgM^b$ mAb (Igh-$6^b$) (Clone AF6-78, IgG1) or anti-mouse $IgM^a$ mAb (Igh-$6^a$) (Clone DS1, IgG1) (Pharmigen, San Diego, CA; Cat. no.Mm-6b-1 P and Igh-6a-1 P). In the second allotype-specific ELISA, wells were coated with purified anti-mouse $IgM^a$ mAb MB86 or anti-mouse $IgM^b$ mAb RS3.1 (a generous gift from Dr A. Grandien, Institut Pasteur, Paris, France).[16] After incubation with titrated concentrations of serum, bound IgM was revealed by purified goat anti-mouse IgM antibodies conjugated to alkaline phosphatase (a generous gift of the Dianova/Jackson Immunoresearch Inc.; Cat. no. 115 055-075/12 99 7). The reference antigens were $IgM^a$ and $IgM^b$ purified from supernatants of the hybridoma cell lines HPC-3/12 and S83.3 (generously provided by Dr A. Grandien, Institut Pasteur, Paris, France).

*Radiolabelling, chemical cross-linking, immunoprecipitation, and polyacrylamide gel electrophoresis (SDS–PAGE)*

C.B-17 thymocytes ($2 \times 10^8$) were labelled with 1 mCi $^{125}I$ using the lactoperoxidase method. Radiolabelled, washed cells were resuspended in either 100 μl serum (approximatley 50 μg IgM) from CD4+ T-cell-induced, IgM-leaky scid mice, or 50 μg monoclonal IgM (control) of an irrelevant specificity, and incubated for 30 min at 4°. Cells were then washed twice and incubated in 0·2 mM dithiobissuccinimidyl propionate (DSP; cleavable under reducing conditions; Pierce Chemicals, Rockford, IL) for 20 min at 20° as described previously.[17] Radiolabelled, cross-linked cells were lysed for 30 min in 0·5 ml NP-40 lysis buffer (10 mM Tris-HCl, 0·15 M NaCl, 1% NP-40, 0·02% $NaN_3$, 0·1 M sucrose, 0·1 PMSF, pH 7·4), followed by centrifugation (8000 ***g***/5 min). Recovered supernatants were dialysed against 0·5 l PBS for 24 hr. Fifty microlitres of rabbit anti-mouse Ig-coated protein G sepharose (Pharmacia, Uppsala, Sweden) were added to the lysates. Lysates were incubated for 18 hr on a rocking platform at 4°. Protein G sepharose was washed three times and boiled for 10 min with and without 10% 2-

mercaptoethanol as a reducing agent. After centrigugation (8000 *g*/5 min), precipitated proteins were separated by 12% SDS-PAGE using approximately 3500 c.p.m./lane. Protein molecular weight markers were obtained from Sigma.

*Statistical analyses*

Statistical analyses performed were the Wilcoxon/Mann–Whitney test of congruence and the Spearman correlation coefficient test.

## RESULTS

### Thymus- , spleen- or lymph node-derived CD4⁺ CD8⁻ T cells from adult dm2 mice repopulate the spleen of young scid mice

Cell sorter-purified CD4⁺CD8⁻ T cells from thymus, spleen or lymph nodes of 3-month-old dm2 (H-2$^d$, L$^{d-}$) donor mice were injected i.v. into 3–4-week-old scid (H-2$^d$, L$^{d+}$) recipient mice. Transplanted scid mice were analysed 10–15 weeks post-transfer. Engraftment of donor T cells into spleens of scid recipients was tested by cytofluorographic analyses and histological examinations. Conforming our previously published data,[15] i.v. injection of $10^5$ splenic CD4⁺CD8⁻ T cells from adult dm2 donor mice engrafted these T cells into the spleens of all scid recipients. Similar data were obtained in transplantation experiments with CD4⁺CD8⁻ T cells from lymph nodes or thymus of adult dm2 mice (Fig. 1): cytofluorographic analyses of SC populations from these transplanted scid mice revealed dm2-derived (L$^{d-}$) CD3⁺CD4⁺CD8⁻ T-cell subsets which represented 2–20% of the recipient's SC population. Numbers of CD4⁺ T cells per spleen varied ($5–50 \times 10^5$ cells/spleen). No differences in the numbers of CD4⁺ T cells engrafted per spleen were found in scid mice transplanted with thymus-, spleen- or lymph node-derived CD4⁺ T cells (Wilcoxon/Mann–Whitney test). Engraftment of T cells was confirmed by growing *in vitro* L$^{d-}$CD3⁺CD4⁺CD8⁻ T-cell lines from spleens of transplanted scid mice (data not shown). These CD4⁺ T-cell lines showed preferential A$^d$-restricted, proliferative reactivity against self determinants as described earlier in a congenic transplantation system.[11,14] Selective and partial repopulation of the T-dependent white pulp areas of spleens of scid recipients by i.v. injected spleen-, lymph node- or thymus-derived CD4⁺CD8⁻ dm2 T cells was apparent in histological examinations. Conclusive evidence was thus obtained that CD4⁺ T cells from spleen, thymus or lymph nodes of adult dm2 mice repopulated the splenic CD4⁺ T-cell compartment of scid mice for more than 3 months.

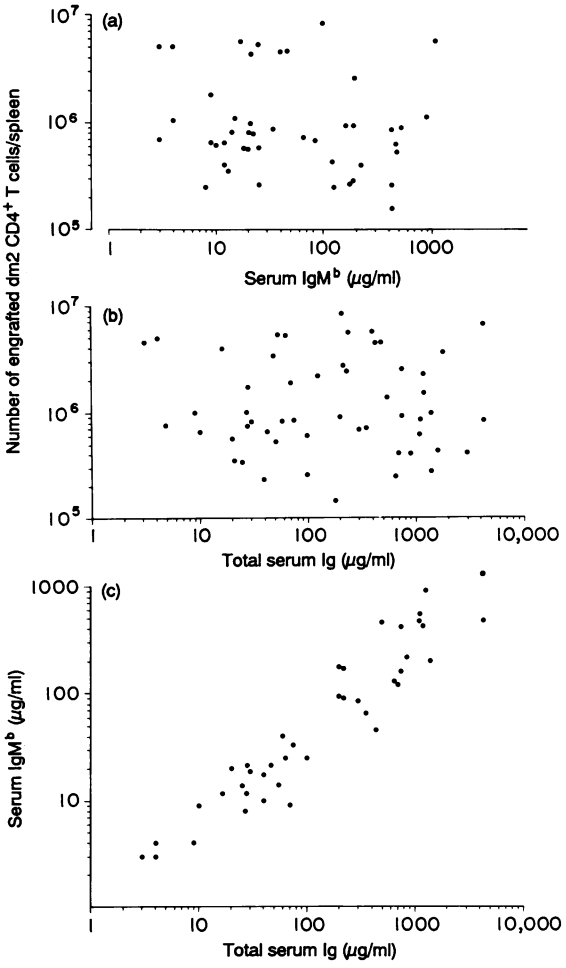


**Figure 1.** Engraftment of CD4⁺ T cells co-reconstitutes functional B cells in scid mice. A panel of 50 individual scid mice transplanted with thymus- or lymph node-derived CD4⁺CD8⁻ dm2 T cells was analysed 3–5 months post-transfer. In transplanted scid mice, the total number of cells/spleen and the fraction (%) of dm2 CD3⁺CD4⁺CD8⁻ T cells/spleen were measured; from these data, the number of engrafted dm2 CD4⁺ T cells/spleen was calculated. In addition, serum IgM$^b$ and IgM$^a$ levels as well as total serum Ig levels were measured by the respective ELISA. All transplanted scid mice tested contained donor-type CD4⁺ T cells in the spleen, and had detectable serum Ig levels. In contrast, more than 30 age- and sex-matched, non-transplanted scid mice did not contain CD3⁺ T cells in the spleen, and total serum Ig levels were always $< 2$ μg/ml (data not shown). The number of donor-type CD4⁺ T cells engrafted per spleen did not correlate either with serum IgM$^b$ levels (a) or with total serum Ig levels (b) (Wilcoxon/Mann–Whitney test). Most of the detected serum Ig was of the IgM isotype (c).

### Transplanted dm2 CD4⁺ T cells rescue IgM-producing B cells in scid mice

Scid mice of our colony below 6 months of age have no detectable Ig in serum.[11] In contrast, we detected Ig in sera of all CD4⁺ T-cell-transplanted, age-matched scid mice 6–15 weeks post-transfer (Figs 1 and 2). In this strain combination, donor and host differ in IgM allotype: dm2 donor mice express the IgM$^a$ allotype, scid (and congenic C.B-17) host mice express the IgM$^b$ allotype. Serum IgM detected in an allotype-specific ELISA in scid recipient mice was predominantly host-derived IgM$^b$ (Figs 1 and 2). IgM concentrations in sera of individual transplanted scid mice were variable, ranging from 1 to 1000 μg/m (Fig. 1). Host-type IgM$^b$ was found in all T-cell-transplanted scid mice. In individual scid recipients, no correlation was found between the number of CD4⁺ T cells engrafted in spleens and either serum IgM$^b$ levels, or total serum Ig levels (Fig. 1a,b).

SC obtained from dm2 CD4⁺ T-cell-transplanted scid mice 10–15 weeks post-transfer were reinjected i.v. into 3-week-old, secondary scid recipients ($3 \times 10^6$ non-fractionated SC/mouse). Donor-type CD4⁺ T cells were recovered from spleens of secondary scid recipient mice analysed 3 months post-transfer. Serially transferred dm2 CD4⁺ T-cell lines expanded extensively in secondary scid recipients for many months. In serial passages of dm2 CD4⁺ T cells through scid mice, serum IgM$^b$ was

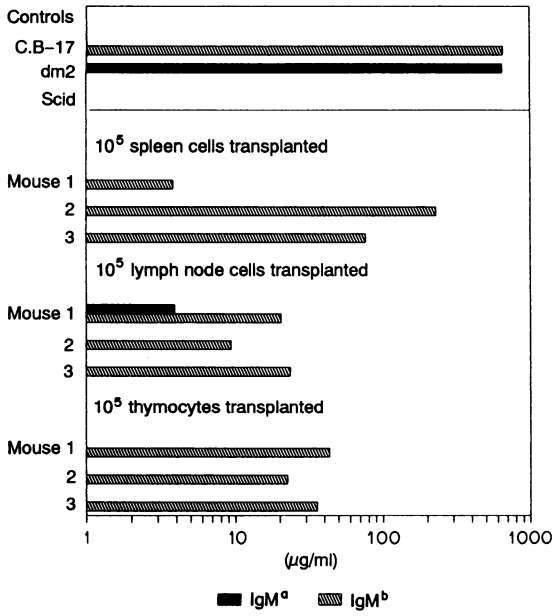


**Figure 2.** Intravenously injected thymic, splenic or lymph node $CD4^+CD8^-$ T cells rescue IgM-producing B cells of the scid host. Young scid mice were transplanted with $10^5$ $CD4^+CD8^-$ T cells purified from spleen, inguinal/axillary lymph nodes or thymus. Twelve to 15 weeks post-transfer, serum concentrations of $IgM^a$ and $IgM^b$ were determined using an allotype-specific ELISA. Controls included sex- and age-matched, non-transplanted scid mice, scid-congenic C.B-17 mice, and dm2 donor mice. Serum IgM measurements of individual transplanted mice are shown.

detectable in all recipients (Fig. 3a). This indicated either that a self-renewing pool of scid B cells was co-transplanted in every passage, or that a new population of scid B cells was rescued following each transplantation.

Host-type IgM was the predominant isotype in serum of most transplanted scid mice, because comparable serum concentrations of total Ig and $IgM^b$ were measured in the ELISA (Fig. 1c). Some exceptions were observed, however, indicating that other isotypes were present in sera of some transplanted scid mice.

We detected no $Ig^+$ cells in re-analyses of cell sorter-purified $CD4^+$ T-cell populations used for transplantations. Occasionally, scid recipients engrafted donor-type $IgM^a$-producing B cells (Figs 2, 3 and 4). Transfers of low numbers of B cells could thus lead to engraftment of host B cells in scid recipients. A transplantation experiment in which purified, splenic dm2 $CD4^+$ T cells were contaminated with transplantable dm2-derived B cells is shown in Fig. 3a. This experiment demonstrated two points: (i) transplanted T cells induced B-cell leakiness in young scid mice in the presence of donor-type, IgM-producing B cells; and (ii), in contrast to dm2 T cells, donor-type B cells could not be serially transferred into secondary or tertiary scid recipients.

### Transfer of low numbers of $CD4^+CD8^-$ thymocytes efficiently induces B-cell leakiness in scid mice

Young scid mice were injected intravenously with titrated numbers of purified spleen- , lymph node- or thymus-derived $CD4^+CD8^-$ dm2 T cells ($10^5$, $10^4$, $10^3$ or $10^2$ cells/mouse). Two-colour cytofluorographic analyses revealed splenic, dm2-derived $CD3^+CD4^+CD8^-$ T-cell subsets in all scid recipients transplanted with $10^3$ or more dm2 $CD4^+$ T cells from thymus, lymph nodes or spleen (data not shown). Periarteriolar T-dependent areas of spleens or scid mice transplanted with low numbers of $CD4^+$ T cells were partially repopulated, confirming histologically T-cell engraftment. No $CD3^+CD4^+$ T cells were found in the spleen of 12 scid mice transplanted with $10^2$ lymph node- or spleen-derived $CD4^+$ T cells.

Serum $IgM^b$ concentrations were higher in scid mice transplanted with thymus- compared to lymph node-derived $CD4^+$ T cells (Fig. 4a,b; Wilcoxon/Mann–Whitney test). A negative correlation was apparent between the number of thymus-derived $CD4^+$ T cells transferred and serum $IgM^b$ levels of scid recipients (Fig. 4b; Wilcoxon/Mann–Whitney test). Transfer of $10^3$ $CD4^+CD8^-$ thymocytes was most efficient in inducing B-cell leakiness in scid mice.

In contrast, intravenous injection of $2 \times 10^7$ non-fractionated dm2 thymocytes into young scid recipients was ineffective in inducing B-cell leakiness in scid mice: 12 weeks post-transfer, serum IgM in transplanted scid mice was predominantly of donor-type (Fig. 3b). Only some recipients showed low levels ($< 10$ μg/ml) of host-type $IgM^b$ in serum (e.g. mice 1, 2, 7, 8 in Fig. 3b). Furthermore, donor-type $IgM^a$ represented the major fraction of serum IgM in secondary scid recipients, 15 weeks after i.v. transfer of $3 \times 10^6$ non-fractionated SC from primary scid recipients of dm2 thymocytes. It is thus evident that $2 \times 10^7$ non-fractionated thymocytes are far less efficient in rescuing scid B cells than $10^3$ purified $CD4^+CD8^-$ dm2 thymocytes.

### Serum antibodies from young scid mice with T-cell-induced B-cell leakiness recognize congeneic immature thymocytes

T-cell populations from primary or secondary lymphoid organs of immunocompetent scid-congenic C.B-17 mice were labelled with sera from scid mice with T-cell-induced B-cell leakiness, and analysed by flow cytometry. Five sera were selected from independent transplantation experiments which contained 300–600 μg/ml $IgM^b$. In 4/5 sera tested, we detected a serological reactivity against $CD4^+$, $CD8^+$ and/or $CD3^{10}$ or $CD3^-$ thymocytes (Fig. 5). We assume that the stained subset represents 'double positive', immature thymocytes. Thus, dm2 $CD4^+CD8^-$ T cells prepared from thymus most efficiently induced B-cell leakiness in scid mice, and antibodies produced by rescued scid B cells reacted with a thymic T-cell subset. Reactivity against resting or activated peripheral T cells from C.B-17 mice was not detected. Some $CD4^+$ T-cell lines expanded *in vitro* from spleens of transplanted scid mice were stained (data not shown).

Bands of 14,000, 28,000, 43,000, 65,000 and 80,000 apparent molecular weight (MW) were revealed by SDS-PAGE analysis of lysates from $^{125}I$-labelled C.B-17 thymocytes reacted with 4/5 sera (described above) from transplanted scid mice with T-cell-induced 'B-cell leakiness' (Fig. 6). Similar patterns were detected in 4/5 tested sera. These proteins were immunoprecipitated neither by control sera from non-transplanted scid mice, nor by 1/5 serum from a transplanted scid mouse. The latter serum contained a serum $IgM^b$ level of 280 μg/ml, but did not stain C.B-17 thymocytes in flow cytometry analyses. Thus 4/5 tested sera from leaky scid mice contained the described reactivity. These bands were only detectable in SDS–PAGE analyses under

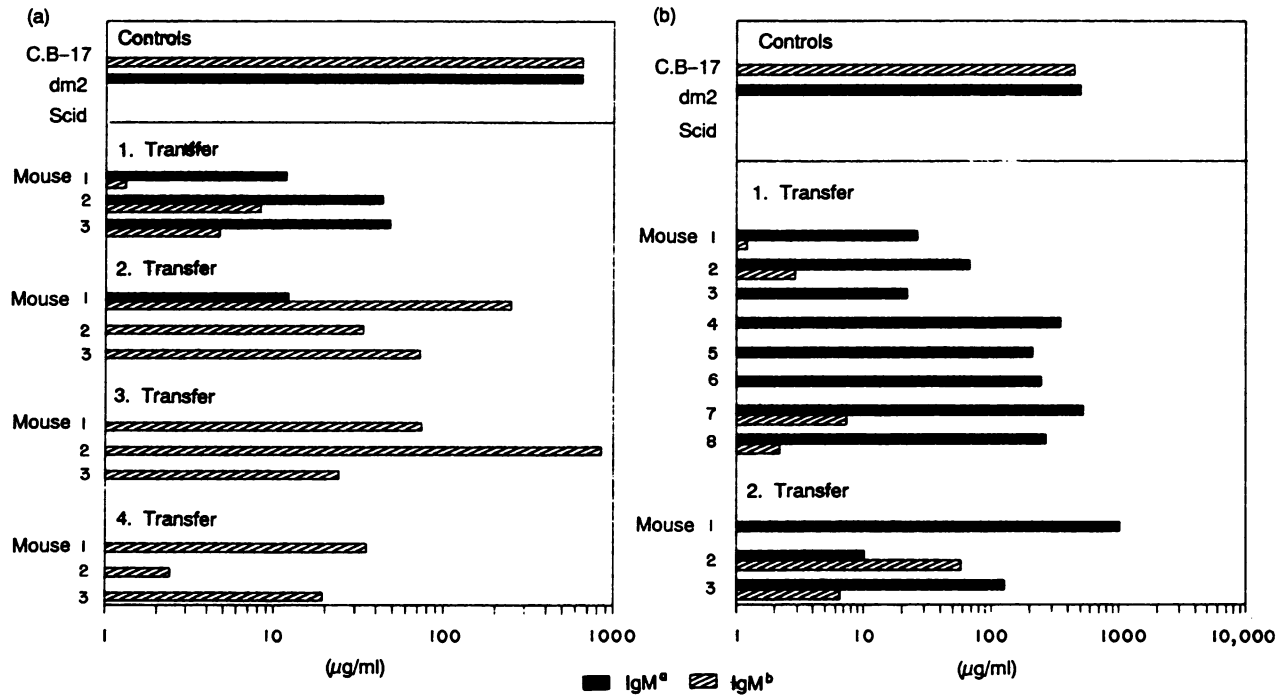


**Figure 3.** (a) Serial passages of dm2-derived CD4⁺ T cells in young scid mice rescue host-derived, IgM-producing B cells. Purified splenic CD4⁺CD8⁻ T cells were injected i.v. into young scid mice. Twelve weeks post-transfer, serum IgM was determined in primary recipient scid mice. Their spleens were removed, and $3 \times 10^6$ non-fractionated spleen cells were reinjected i.v. into young secondary scid recipients. Secondary recipients were analysed 3 months post-transfer. Four different transfers of three individual T-cell lines covering an observation period of 14 months are shown. (b) Transplantation of large numbers of non-fractionated dm2 thymocytes into young scid mice. Non-fractionated thymocytes from adult dm2 donor mice were injected i.v. into young scid mice ($2 \times 10^7$ cells/mouse). Recipient scid mice were analysed 3 months post-transfer. Non-fractionated SC from recipient scid mice 1–3 were re-injected into secondary recipient scid mice ($3 \times 10^6$ cells/mouse) which were analysed 15 weeks post-transfer. Control mice as in Fig. 2.

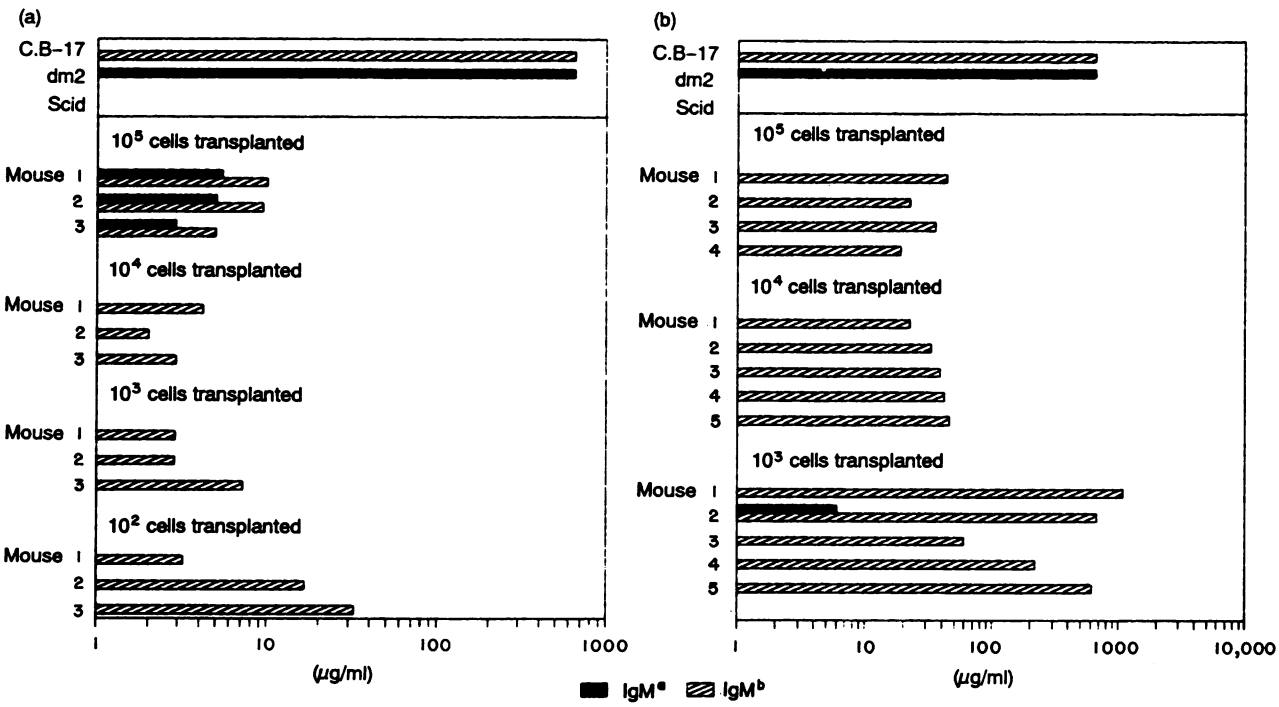


**Figure 4.** Intravenous injection of limiting numbers of purified CD4⁺CD8⁻ T cells from adult dm2 donor mice induces B-cell leakiness in young scid recipient mice. Titrated numbers of CD4⁺CD8⁻ T cells purified from lymph node (a) or thymus (b) of adult dm2 donor mice were injected i.v. into young scid recipients. Serum $IgM^a$ and $IgM^b$ concentrations were measured 12–15 months post-transfer. Control mice as in Fig. 2.

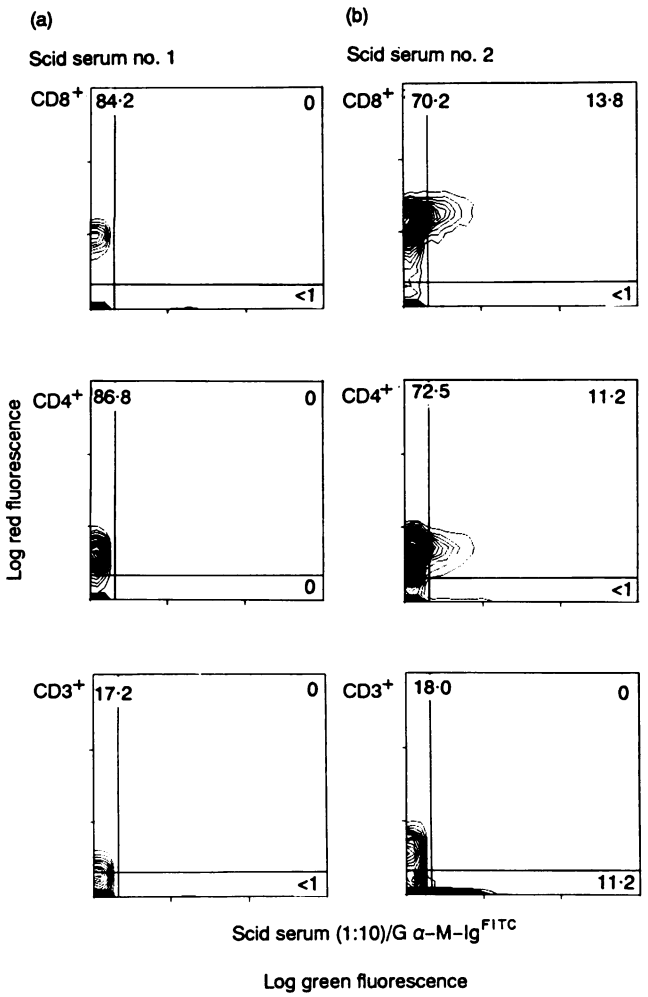


**Figure 5.** Antibodies in sera from transplanted young scid mice with T-cell-induced 'B-cell leakiness' react with congenic double-positive thymocytes. Thymocytes from adult, scid-congenic C.B-17 mice were incubated with (1:10 diluted) serum from scid mice with T-cell-induced 'B-cell leakiness'. Bound immunoglobulin was revealed by FITC-conjugated goat anti-mouse antiserum. Labelled cells were analysed by flow cytometry. Four out of five tested sera reacted with $CD3^-$ or $CD3^{low}$, 'double-positive' ($CD8^+CD4^+$) thymocytes (b). The serum from a transplanted leaky scid mouse (with a serum-$IgM^b$ level of 280 $\mu$g/ml) in which no such reactivity was detected is shown in (a) as a negative control.

reducing conditions; analyses under non-reducing conditions showed only a single 14,000 MW band. These data suggest that T-cell transfer rescues anti-T-cell 'autoantibody'-producing B cells in scid mice.

## DISCUSSION

Neither serum Ig, nor $Ig^+$ B cells or $CD3^+$ T cells, are detectable in the spleen, thymus, lymph nodes or peritoneal cavity of young scid mice below 6 months of age. Intravenous injection of $10^3$–$10^5$ purified thymic, splenic or lymph node $CD3^+CD4^+CD8^-$ T cells from adult dm2 donor mice into young scid mice leads to partial and selective reconstitution of $CD4^+$ T-cell populations in splenic T-dependent areas. Engraftment of $CD4^+$ T cells in scid mice is always accompanied by rescue of scid-derived, IgM-producing B cells.

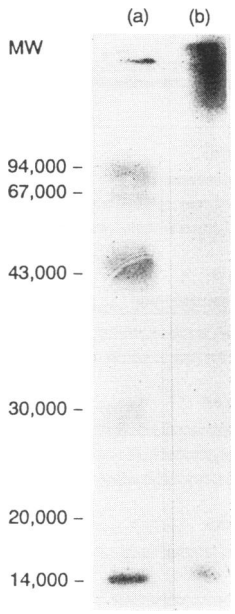


**Figure 6.** Immunoprecipitation of surface proteins from congeneic thymocytes using self-reactive IgM antibodies from leaky scid mice. SDS–PAGE and autoradiography of material from $^{125}I$-labelled C.B-17 thymocytes reacted with IgM-containing serum from transplanted scid mice with T-cell-induced B-cell leakiness (lane a, reducing conditions; lane b, non-reducing conditions). Arrows indicate positions of revealed bands. Numbers represent molecular weight (MW).

The molecular basis of the leaky scid phenomenon is unclear. Functional T and B cells may be generated in scid mice by infrequent productive rearrangements at different antigen receptor-encoding gene loci.[5] Alternatively, evidence for reversion of scid recombinase activity has come from studies on TcR and Ig re-arrangement patterns of leaky scid lymphocytes.[3,18] In addition, a rare event (e.g. specific activation and/or growth promotion) seems essential to clonally expand these lymphocytes above the detection threshold. All scid mice transplanted at 3–4 weeks of age with purified $CD4^+$ T cells developed measurable serum Ig concentrations 2–4 months post-transfer. None of the sex- and age-matched, non-transplanted scid mice tested were $Ig^+$. This implies that all young scid mice contain inducible B cells that can be rescued. These data, as well as other published evidence,[12-14] are compatible with the idea that scid mice continuously generate low numbers of potentially competent, antigen receptor-expressing lymphocytes which need either a 'relief from suppression' or a particular stimulus to clonally expand. The possiblity of 'relief from suppression' is suggested by the observation that young scid mice develop serum Ig titres following natural killer (NK) cell suppression after treatment with anti-asialo-GM1 antibodies.[19] A positive growth stimulus can apparently be provided by mAb with particular specificities,[12] neonatal thymocytes,[13] self-reactive $CD4^+$ T-cell clones,[14] congenic and semi-allogeneic $CD4^+$ T cells from various lymphoid organs (this paper) or microbial antigens (in 'spontaneously' leaky scid mice in non-SPF environments).[3]

The nature and the physiological importance of the stimulus involved in this type of T–B co-operation in leaky scid mice is unknown. It is possible that either transplanted T cells rescue

scid-derived B cells, or scid-derived B cells may select particular T-cell subsets from the transplanted T-cell population for engraftment. These interactions may operate by direct cell contact or by cytokines. Oligoclonal T–B networks might be constituted *in vivo* in transplanted, leaky scid mice by repertoire selections involving specific recognition of antigen receptor idiotypes. Some of the available experimental data are compatible with this idea.[12-14] Alternatively, cytokines or antibodies with 'cytokine-like' activity may play a role in the engraftment of T cells and/or rescue of B cells. Antibodies with 'cytokine-like' activity have been described, e.g. antibodies with IL-3-like activity in lpr mice.[20] We have previously shown that engraftment of $CD8^+$ T cells in scid mice requires continuous substitution of recipients with IL-2.[15] Treatment of non-transplanted young scid mice with IL-2 did not rescue T or B cells. Cytokines from transplanted $CD4^+$ T cells may stimulate mature or precursor B cells in scid mice, or may down-regulate suppressor cells in scid mice. The observation of T-cell-induced B-cell leakiness in scid mice raises the question of the role of B cells in peripheral T-cell expansion. Transgenic mice with deleted Ig$\mu$ gene expression contain no B cells but apparently normal numbers of T cells.[21] This suggests that B cells are not an essential prerequisite for T-cell repopulation of peripheral lymphoid organs.

Serum Ig concentrations varied 1000-fold in scid mice with $CD4^+$ T-cell-induced leakiness (Fig. 1). Variations in serum $IgM^b$ levels were also observed when $10^5$ cloned autoreactive $CD4^+$ T cells were injected into young scid mice.[14] Variability in serum IgM levels thus seems inherent in the scid host B-cell response. We have observed an inverse correlation between scid serum $IgM^b$ level and number as well as type of injected $CD4^+$ T cells, i.e. transfer of low numbers of $CD4^+CD8^-$ thymic T cells induced high $IgM^b$ serum levels. Thus, the inducing stimulus also has an influence on the magnitude of the scid B-cell response. Scid mice may generate only a limited number of potentially functional B cells of a given specificity in the course of a limited time interval post-transfer. This may result in variable numbers of B-cell clones that can be rescued by a given stimulus. In addition, the number and life-span of Ig-producing cells developing from a given B-cell clone and different clearance rates of Ig from the circulation may contribute to the extreme variability of serum Ig levels in leaky scid mice.

Bone marrow from scid mice contains normal frequencies of ($B220^+$, Thy-$1^{lo}$, $IgM^-$) pro-B cells.[22] The progeny of pro-B cells, i.e. $B220^+$, Thy-$1^-$, $IgM^-$ pre-B cells, are absent from scid bone marrow.[3,22] Transformation of scid bone marrow cells with Abelson murine leukaemia virus resulted in pre-B-cell lines with abnormally re-arranged IgH genes.[23] Cells with the pre-B phenotype and abnormally rearranged IgH genes were recovered from long-term scid bone marrow cultures set up under growth conditions selective for B cells.[24] A normal pool of progenitor cells of the B-cell lineage is thus present in scid marrow but differentiated progeny cells are rapidly eliminated *in vivo* because of defective Ig re-arrangements. The developmental fate of the occasional scid B cells which succeed in productively re-arranging IgH and IgL gene segments is unknown.

Serum Ig in leaky scid mice was mostly of the IgM isotype. Serum levels of Ig of other isotypes were not determined because allotypic markers for other Ig isotypes are not defined in our donor/host strain combination. It is important to discriminate between donor- and host-type Ig in view of the observation that low numbers of injected dm2 B cells engraft, clonally expand and produce substantial levels of IgM (Figs. 2–4). In most transplanted scid mice, measured serum concentrations of total Ig and $IgM^b$ were of similar magnitude, suggesting that isotypes other than IgM represented minor fractions of serum Ig. Immunoglobulin of the (T-dependent) IgG1 isotype was reported to be present in serum of scid mice with T-cell-induced, but not with 'spontaneous' B-cell leakiness.[13]

Injection of neonatal but not adult thymocytes was reported to induce B-cell leakiness in scid mice.[13] This study confirms that i.v. injection of $2 \times 10^7$ non-fractionated thymocytes from adult dm2 donor mice is poor in inducing B-cell leakiness in young scid recipients. In contrast, transfer of low numbers ($10^3$–$10^5$ cells/mouse) of purified $CD4^+CD8^-$ thymocytes from adult donor mice efficiently induced B-cell leakiness. Antibodies produced in leaky scid mice stained immature $CD3^-$ $CD4^+CD8^+$ thymocytes. Protein bands were revealed by SDS–PAGE analyses of radioactive material from congeneic thymocytes immunoprecipitated with 'leaky' antibodies. The nature of the precipitated bands is unknown. Thymocytes may be a preferred, but not the exclusive, T-cell type to rescue B cells in scid mice.

dm2 B cells initiated IgM secretion upon transfer to scid mice. Scid B cells were rescued by dm2 $CD4^+$ T cells in the presence as well as the absence of donor-type B-cell engraftment. Donor-type B cells therefore did not interfere with B-cell rescue in young scid mice, i.e. we did not observe 'feedback competition' among B-cell subpopulations, which has been described in other transplantion experiments in scid mice.[13,25] Neither engrafted donor-type B cells nor rescued scid-type B cells rescued scid-derived ($L^{d+}$) $CD3^+$ T cells in the course of a 3–4-month observation period post-transfer.

The described murine model may offer the possibility to elucidate the role of T–B interactions in establishing lymphocyte populations in peripheral lymphoid organs. The characterization of T-cell-induced B-cell leakiness in scid mice, in the course of which autoreactive antibodies are generated, might offer the opportunity to serologically define immunogenic self-components.

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