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Immunologic Characterization of CD7-Deficient Mice

David M. Lee,* Herman F. Staats,*† John S. Sundy,* Dhavalkumar D. Patel,*† Gregory D. Sempowski,* Richard M. Scearce,* Dawn M. Jones,* and Barton F. Haynes2*†

Human CD7 is an Ig superfamily molecule that is expressed on mature T and NK lymphocytes. Although in vitro studies have suggested a role for CD7 in lymphoid development and function, the exact function of CD7 in vivo has remained elusive. One patient has been reported with SCID syndrome attributed to CD7 deficiency. To study in vivo functions of CD7, we have generated CD7-deficient mice and assessed their lymphoid development and function. CD7-deficient mice were viable, had normal peripheral blood and spleen lymphocyte numbers, and had normal specific Ab responses with Ag-driven Ig isotype switching. Thymocyte numbers were normal in 4-wk-old, 6-mo-old, and 1-yr-old CD7-deficient mice, but in 3-mo-old CD7-deficient mice, total thymocyte numbers were significantly increased by 60% (p < 0.02) compared with normal age-matched +/- littermates. CD7-deficient splenocytes proliferated normally in response to various mitogens, including PHA, anti-CD3, Con A, and LPS. While NK cell numbers and cytolytic activity to YAC targets were normal, CD7-deficient mice had lower Ag-induced MHC class I-restricted CTL activity against OVA-transfected target cells than did +/- control mice. Thus, CD7-deficient mice did not have a SCID syndrome, but rather had transient increases in thymocyte numbers at 3 mo of age and altered splenocyte Ag-specific CTL effector cell activity. These data suggest a role for CD7 in regulating intrathymic T cell development and in mediating CTL effector function.


Human CD7 is a 40-kDa member of the Ig gene superfamily that is expressed on mature T and NK cells and on T, B, NK, and myeloid lineage precursors in bone marrow (1–8). CD7 is expressed early in T lymphocyte development before CD2, CD3, TCR, CD4, and CD8 (8, 9). Although the function of CD7 is unknown, studies suggest that CD7 may be involved in T and NK cell activation and/or adhesion. In TCRαβ T cells, cross-linking of CD7 leads to rapid increases in intracellular calcium concentrations (10, 11), induces CD7 cytoplasmic domain association with phosphotidylinositol-3-kinase (12, 13), synergizes with submutigenic amounts of CD3 mAbs to induce T cell activation (14, 15), modulates T cell adhesion (16), and increases T cell IL-2 production and IL-2 receptor expression (15).

In TCRγδ T cells, cross-linking of CD7 leads to cell activation and induction of TNF-α, TNF-β, and granulocyte-macrophage CSF mRNA (17). In NK cells, cross-linking of CD7 leads to cell proliferation, IFN-γ production, increased ability to kill NK target cells, and increased cell adhesion to fibronectin via β1 integrins (6, 18). In bone marrow T and myeloid progenitor cells, cross-linking of CD7 leads to granulocyte-macrophage CSF production (19). Interestingly, a patient has been described with SCID syndrome whose lymphocytes were CD7 negative (20).

Although the murine CD7 (mCD7) cDNA and gene have been cloned (21–23), and its message expression has been demonstrated in lymphoid organs, little is known about mCD7 tissue expression and function due, in part, to lack of murine anti-CD7 mAbs. To probe in vivo roles of CD7 in murine immune system development and function, we have constructed and characterized CD7-deficient mice. We found that CD7-deficient mice did not have a SCID syndrome, but rather had a transient increase in thymocyte number at 3 mo of age and had decreased splenic Ag-specific CTL effector activity against OVA-transfected EG.7 ova cells.

Materials and Methods

Construction of CD7-deficient mice

Disruption of the mCD7 gene was accomplished in 129 strain embryonic stem (ES) cells using described methods (24). A homologous recombination cassette was constructed using the pPNT vector (25) by inserting 1.6 kb of the mCD7 gene ending midway through exon 3 (corresponding to the predicted membrane proximal extracellular domain), with the addition of an in-frame stop codon 5′ of the neo’ gene (Fig. 1A). This fragment was generated using PCR primers containing NotI restriction sites on both ends (5′ primer, 5′-ATAAGAATGCGGCCGCTGTTGTAAGCAGATGGCTGCT-3′, and 3′ primer, 5′-ATAAGAATTTCGCGCCGCTTATGGTAGCCAGATGGCGCTG-3′) with the addition of an in-frame stop codon within exon 3. A 3′ fragment of the mouse CD7 gene, beginning midway through intron 3 at the EcoRI restriction endonuclease site and extending to 2.5 kb 3′ of exon 4, was inserted into the EcoRI site of the pPNT vector 3′ of the neo’ gene and 5′ of the herpes thymidine kinase gene. This fragment was derived from subcloned genomic DNA. ES cell clones with homologous recombination of the disrupted allele of CD7 were identified via Southern blot analysis (26) of EcoRI-digested genomic DNA due to a 2-kb RFLP resulting from the insertion of the neo’ gene. The probe used for Southern blotting was a 400-bp PCR-generated fragment of genomic DNA immediately adjacent 5′ to exon 1 (used in the homologous recombinant construct). The sequence of the primers used to generate this probe were: 5′ primer, 5′-TCAACTCCTCCCCCGCCTTTCT-3′, and 3′ primer, 5′-AGACATGCGCTGCTAGTCA-3′.

Chimeric mice were generated by insertion of 129 strain ES cells into C57BL/6 recipient embryos (24). These chimeric offspring were backcrossed to C57BL/6 mice, and germline transmission of the disrupted CD7 allele was identified by Southern blot analysis of DNA purified from tail tissue and digested with EcoRI endonuclease. Tail DNA was extracted by incubating tail tissue in buffer containing 50 mM Tris, pH 8.0, 100 mM

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3 Abbreviations used in this paper: mCD7, murine CD7; ES, embryonic stem; DP, double positive.
EDTA, 0.5% SDS, and 1 mg/ml proteinase K at 55°C overnight. The samples were phenol/chloroform extracted and ethanol precipitated. Heterozygous offspring were intercrossed to generate CD7-deficient (2/2) and control (1/1) mice. The data in Tables I and II and Figures 1 and 2 were obtained using the initial CD7-deficient mice obtained from one or two backcrosses. Phenotyping analysis of thymocyte number in CD7-deficient mice backcrossed to C57BL/6 mice five times have been performed and showed increased thymocyte numbers identical to those data in Figure 2. All functional cellular assays in this paper were performed in CD7-deficient mice backcrossed onto C5BL/6 mice at least five times.

**Abs, reagents, and flow cytometry**

mAbs Thy1.2-FITC (Anti-Thy-1; Becton Dickinson, Mountain View, CA), Ly5-FITC (anti-B220, PharMingen, San Diego, CA), Ly6-FITC (anti-CD8, Becton Dickinson), and L3T4-PE (anti-CD4, Becton Dickinson) CD8α-TRI (Caltag, South San Francisco, CA) were used at saturating titers for flow cytometry. mAbs PK136-FITC (anti-NK1.1; ATCC HB191 (American Type Culture Collection, Manassas, VA)) and OX-12 (anti-rat κ chain) (Sera-Labs, Crawley Down, Sussex, U.K.) were produced from hybridomas cultured in serum-free medium (SFM; Life Technologies, Grand Island, NY) and purified using affinity chromatography over a Staph protein A/G column (Pierce, Rockford, NY). Purified PK136 and OX-12 were subsequently fluorescein conjugated, and saturating titers were determined. mAbs E13 (27, 28) (anti-granulocyte lineage), Ter 119 (29) (anti-erythroid lineage), and F4/80 (ATCC HB 198) (anti-macrophage subset) were used in saturating concentrations in flow cytometry (30). Cell suspensions were prepared and used as described (30, 31).

**Northern blot analysis of CD7 mRNA expression**

Total cellular RNA was collected from control and CD7-deficient mice by lysis of freshly teased splenocytes and thymocytes using the TRizol (Life Technologies) reagent per manufacturer protocol. Total RNA (30 μg) was run on a 1.2% formaldehyde-containing agarose gel in 3-(N-morpholino)propanesulfonic acid buffer as previously described (26). After transfer to

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**FIGURE 1.** Construction and identification of CD7-deficient mice. A, Schematic representation of two regions of mouse CD7 (mCD7) subcloned into the plasmid pPNT. pPNT encoded the neomycin and thymidine kinase genes used for positive and negative selection of transfected 129 strain ES cells. A null allele of mCD7 was accomplished by interrupting the mCD7 gene sequence in the 5’ region of exon 3 (before the sequence encoding the transmembrane region) through insertion of a stop codon and by insertion of the neomycin resistance gene. The 3’ region of exon 3 was not included in this plasmid. The solid lines illustrate the region of mCD7 interrupted by the neo’ gene. Lower panel, structural organization of the mCD7 gene. Hatched lines represent regions where homologous recombination is required. Selected restriction sites are labeled: H3 signifies HindIII, EcoRI, BamHI, XbaI, and NdeI. Germine transmission and subsequent allele-carrying offspring were identified by genomic Southern blot of tail DNA. B, Genomic Southern blot of tail DNA from three offspring digested with the restriction endonuclease EcoRI. Mouse number and genotype are labeled. + signifies wild-type CD7, and − signifies disrupted CD7. Lack of CD7 RNA was verified in Northern blot analysis of total RNA isolated from splenocytes and thymocytes of control (+/+) and CD7-deficient mice and probed with mouse CD7 cDNA (C).
nylon membrane and UV cross-linking, this RNA was probed with mouse CD7 cDNA using QuickHyb (Stratagene, La Jolla, CA) per manufacturer protocol.

**Splenocyte mitogenic responses**

For these mitogenic proliferation assays, single-cell suspensions of freshly teased and Ficoll-Hypaque isolated splenocytes (Fico-Lite; Atlanta Biologics, Atlanta, GA) were adjusted to 1 × 10^6 cells/ml in RPMI 1640 supplemented with 10% FCS (HyClone, Logan, UT) 5 × 10^{-5} M 2-ME, 10 µg/ml gentamicin. Cells (1 × 10^5; 100 µl) were incubated in the presence of either medium, anti-CD3 (2C11; Ref. 32), anti-CD3 plus anti-CD28 (37.5; Ref. 33), PHA (Murex Diagnostics Ltd., Dartford, U.K.), Con A (Sigma, St. Louis, MO), or LPS (Sigma) in round-bottom 96-well microtiter plates in triplicate and incubated at 37°C in a 10% CO2 in an air-humidified environment for 3 (PHA) or 4 days. Four hours before harvesting, 0.4 µCi [3H]thymidine in 20 µl medium was added to each well.

**Thymocyte mitogenic responses**

For these mitogenic proliferation assays, single-cell suspensions of freshly teased thymocytes were adjusted to 1 × 10^6 cells/ml in RPMI 1640 supplemented with 10% FCS (HyClone), 5 × 10^{-5} M 2-ME, 5 ng/ml rIL-2 (R&D Systems, Minneapolis, MN), and 10 µg/ml gentamicin. Cells (1 × 10^5; 100 µl) were incubated in the presence of either medium, anti-CD3 (2C11; Ref. 32), anti-CD3 plus anti-CD28 (37.5; Ref. 33), PHA (Murex Diagnostics Ltd., Dartford, U.K.), Con A (Sigma, St. Louis, MO), or LPS (Sigma) in round-bottom 96-well microtiter plates in triplicate and incubated at 37°C in a 10% CO2 in an air-humidified environment for 3 (PHA) or 4 days. Four hours before harvesting, 0.4 µCi [3H]thymidine in 20 µl medium was added to each well.

**Tetanus toxoid immunizations**

Mice were immunized with 50 µg tetanus toxoid (Wyeth Ayerst Laboratories, Marietta, PA) in CFA (Sigma) on day 0 and boosted with 50 µg tetanus toxoid in incomplete Freund’s adjuvant (Sigma) on day 28 or 35 after the primary immunization. Mice were anesthetized with ketamine/xylazine for all immunizations.

**ELISA**

ELISA was used to determine the presence of anti-tetanus toxoid Abs in serum samples. TT was suspended in CBC buffer (15 mM Na_2CO_3, 35 mM NaHCO_3, pH 9.6) at a concentration of 3 µg/ml and plated to 96-well microtiter plates (model 3590; Costar, Cambridge, MA) at 50 µl/well. After overnight incubation at 4°C, the contents of the wells were discarded, and blocking buffer (CBC with 3% nonfat dry milk) was added to 200 µl/well. All samples were diluted in serum diluent (PBS, 2.5% BSA, 2.5% nonfat dry milk, 5% normal goat serum, 0.1% sodium azide, and 0.05% Tween-20) and added to ELISA plates at 100 µl/well. After overnight incubation at 4°C, plates were washed four times with ELISA wash buffer before the detection Ab was added. Alkaline phosphatase-conjugated, goat anti-mouse IgG, IgA, or IgM (Southern Biotechnology Associates, Birmingham, AL) was diluted 1:1,000 (in PBS, 0.05% Tween-20, and 0.5% BSA) and used as the detection Ab (100 µl/well). After incubation at room temperature for 3 h, plates were washed four times with ELISA wash buffer and reacted with the alkaline phosphatase substrate p-nitrophenyl phosphate. After a 10-min incubation, plates were read at 405 nm on a Titertek Multiscan Plus plate reader.

**NK cell and CTL activity**

NK cell cytotoxicity was determined with a 4-h 51Cr-release assay using YAC-1 mouse lymphoma cells as targets. Mice were injected i.p. with 100 µg polycl(IgC) (Sigma) 24 h before splenocyte harvest. The percentage of 51Cr release from target cells was calculated using the following formula: % specific lysis = 100 × (cpm experimental release – cpm spontaneous release)/(cpm detergent lysis – cpm spontaneous release).

Ag-specific CTL effector activity was assessed using splenocytes from OVA peptide-primed mice as previously described (34, 35). CD7+/+ and C57BL/6 control mice were immunized intranasally with 60 µg OVA peptide plus 0.5 µg cholera toxin subunit (List Biologics, Campbell, CA) in 15 µl of PBS. This immunization was administered four times at 7-day intervals. Fourteen days after the final immunization, splenic mononuclear cells were placed in culture at 2 × 10^6 cells/ml with 2 × 10^5 cells/ml γ-irradiated EG7ova (35) stimulator cells for 6 days. After culture, CTL activity was assessed with a 4-h 51Cr release assay using EG7ova (35), EL-4 (ATCC TIB 39), or P815 (ATCC TIB 64) mouse lymphoma cells as targets. The percentage of 51Cr release from target cells was calculated using the following formula: % specific lysis = 100 × (cpm experimental release – cpm spontaneous release)/(cpm detergent lysis – cpm spontaneous release). Lytic units were defined as the number of splenocytes required to achieve 30% specific lysis as previously reported (36).

**Cytokine quantitation**

Quantities of cytokines present in culture supernatants were assessed using commercial sandwich cytokine ELISA assays per manufacturer protocol. IL-4, IL-10, and IFN-γ ELISA assays were obtained from Pharmingen (San Diego, CA), while TNF-α was obtained from Genzyme Corp. (Cambridge, MA).

[3H]Thymidine incorporation assays

Ag-specific proliferative responses were assessed using splenocytes from tetanus toxoid-primed mice in [3H]thymidine incorporation assays. For these assays, single-cell suspensions of freshly teased spleen cells were incubated with 10% FCS. Cells were incubated in the presence of either medium or tetanus toxoid at 100 µg/ml, 10 µg/ml, or 1 µg/ml in round-bottom 96-well microtiter plates in triplicate and incubated at 37°C in a 10% CO2 in a humidified air environment for 4 days. Twelve hours before harvesting, 0.4 µCi [3H]thymidine was added to each well. Culture supernatants from identical conditions were harvested for cytokine analysis.

**Results**

**Construction and initial characterization of CD7-deficient mice**

DNA from mice homozygous for the homologous recombination CD7 gene was probed and characterized by Southern blot analysis (Fig. 1B). Abundance of CD7 expression was documented by Northern blot analysis of thymus and spleen RNA, showing CD7 mRNA present in normal, +/+ mice and absent in CD7-deficient, −/− mice (Fig. 1C). These results were confirmed by RT-PCR analysis, in which no full-length CD7 cDNA was detectable (not shown). CD7-deficient mice bred normally, and histologic analysis of autopsied tissues (thymus, spleen, lymph node, brain, stomach, small intestine, large intestine, heart, skeletal muscle, salivary gland, kidney, and pancreas) was normal (not shown). In particular, thymic cortical and medullary areas and B cell follicles and germinal centers in lymph node and spleen were present and normal (not shown). Thus, CD7-deficient mice did not have a SCID syndrome.
Next, the absolute numbers of lymphoid subsets in CD7-deficient, −/− and normal, +/+ littermate controls in bone marrow, spleen, peripheral blood, and thymus were determined (Fig. 2A). Whereas spleen, peripheral blood, and bone marrow cell numbers were normal in CD7-deficient mice, thymocyte numbers in 3-month mice were increased 60% compared with +/+ normal littermates \((77 \times 10^6 \pm 9 \times 10^6)\) CD7-deficient thymocytes vs \(48 \times 10^6 \pm 6 \times 10^6\) normal +/+ thymocytes; \(p < 0.02, n = 6\). These data were obtained in the initial CD7-deficient mice backcrossed to C57BL/6 mice one or two times, and similar results were also
obtained in CD7-deficient mice backcrossed to C57BL/6 mice five times (not shown). Interestingly, the increase in CD7-deficient thymocyte numbers was transient, since 4-wk-old, 6-mo-old, and 12-mo-old CD7-deficient mice did not have increased thymocyte numbers compared with control mice (Fig. 2B). Subset analysis of thymocytes revealed that the increase in thymocytes at 3 mo of age was due to selective increased number of CD4+CD8+ (double-positive (DP)) thymocytes (67 × 10^6 ± 8 × 10^6 CD7-deficient DP thymocytes vs 41 × 10^6 ± 6 × 10^6 control mouse DP thymocytes; n = 6, p < 0.02) (Fig. 2C). Analysis of lymphoid subsets in spleen and peripheral blood showed no differences in percentage or absolute number of T cells, B cells, monocytes/macrophages, or NK cells (Tables I and II); nor were differences noted in bone marrow granulocyte-, erythroid-, or B-lineage subpopulations (not shown).

In vivo Ab responses to Ag

CD28 is an Ig superfamily costimulatory molecule that, like CD7, associates with PI-3 kinase (37–40). CD28-deficient mice, like CD7-deficient mice, developed normally and had normal lymphoid organs (41). However, CD28-deficient mice had defective B cell responses to Ag with a deficiency of Ig isotype switching (41). In CD7-deficient mice, total serum Ig levels and serum Ig subclass levels (IgG, IgG2a, IgG2b, IgG3, IgA, and IgM) were normal (not shown). In addition, when anti-tetanus toxoid-specific Ab responses were tested, no differences were noted in serum anti-tetanus toxoid Ig isotypes between CD7-deficient and control mice (Fig. 3). Thus, in CD7-deficient mice, no defects in T-dependent anti-tetanus toxoid B cell Ig responses were apparent.

\[^{3}H\]Thymidine incorporation and cytokine production assays of CD7-deficient splenocytes and thymocytes

Since previous reports demonstrated a role for human CD7 as a comitogen for T lymphocyte mitogenic responses (14, 15), we tested \[^{3}H\]thymidine incorporation responses by CD7-deficient splenocytes (to Con A, PHA, CD3, and CD3 + CD28 mAbs) and thymocytes (to Con A, PHA, and CD3 + rIL-2). In addition, proliferative responses to tetanus toxoid by splenocytes from CD7-deficient and control mice previously immunized with tetanus toxoid were assessed. Unlike the T cell-triggering abnormalities seen in CD28-deficient mice (41), we found no differences in \[^{3}H\]thymidine incorporation between CD7-deficient and control mouse splenocytes (Fig. 4A) or thymocytes (Fig. 4B) in mitogen-stimulated or TCR-mediated T cell activation assays.

Since work has demonstrated a role for CD7 in stimulation of cytokine production in human T cells (17), cytokine levels in culture supernatants from mitogen- and Ag-stimulated splenocyte and thymocyte cultures described above were determined. Analysis of IL-4, IL-10, TNF-\(\alpha\), and IFN-\(\gamma\) supernatant levels demonstrated a significant difference between CD7-deficient and control mice only with IFN-\(\gamma\) produced in the presence of tetanus toxoid-primed splenocytes stimulated in vitro by tetanus toxoid (0.7 ± 0.3 ng/ml vs 3.24 ± 0.7 ng/ml; p < 0.02, n = 6) (Fig. 4, C and D; data for IL-4, IL-10, and thymocytes are not shown). IFN-\(\gamma\) induced by CD3 stimulation of CD7-deficient splenocytes was also less than control; however, this difference did not reach statistical significance (IFN-\(\gamma\) in CD7-deficient splenocytes, p = 0.06).

NK cell activity

Since CD7 has been implicated in regulating NK cell function (6, 18), we tested the ability of CD7-deficient splenocytes to lyse \(^{51}\)Cr-labeled YAC targets. We found that splenocytes from CD7-deficient mice were able to lyse YAC targets to the same degree as age-matched control mice (Fig. 5), demonstrating that functional NK cells were present in CD7-deficient mice.

Ag-specific MHC class I-restricted CTL activity

Next, we assessed the ability of CD7-deficient mice to generate CTL activity to the E.G7ova cell line expressing chicken OVA, after immunization of CD7-deficient mice with an OVA peptide in a system that has previously been demonstrated to generate CD8\(^{+}\), MHC class I-restricted CTL in MHC H\(_{2}\)D\(^{b}\) C57BL/6 mice (34). We found that the CTL lytic activity in CD7-deficient mouse splenocytes was significantly decreased compared with control splenocytes at multiple E:T ratios (Fig. 6). Interestingly, supernatants of splenocytes after 7 days of in vitro culture with irradiated OVA-expressing cells showed equal IFN-\(\gamma\) levels in splenocytes from CD7-deficient and control mice (not shown). Thus, deficiencies in IFN-\(\gamma\) production in OVA-stimulated splenocyte bulk cultures cannot explain the decrease in ova-specific CTL activity in CD7-deficient splenocytes vs normal +/- mice.

Discussion

In this paper, we describe construction and initial immunologic characterization of CD7-deficient mice. These mice were healthy with normal lymphoid development. Thymocyte numbers were transiently elevated by 60% in 3-mo-old mice but not in 4-wk-old,

FIGURE 3. Time course and magnitude of B cell Ig responses to tetanus toxoid immunization. Shown are the reciprocal titers of IgM (A) and IgG (B) isotype responses to tetanus toxoid following immunization in control +/+ and CD7-deficient −/− mice. Mice were immunized in Freund’s adjuvant at day 0 and day 28 (arrows). Data are plotted as log\(_{10}\) reciprocal titers (A) (Fig. 4, A and C) and IgG (B) isotype of the antitetanus toxoid Ab response analyzed on postimmunization day 56 in ELISA that determined the quantity of IgG1, IgG2a, IgG2b, or IgG3 present. Data are plotted as log\(_{10}\) reciprocal titers ± SEM (n = 4 +/- and 5 −/−). C, IgG isotype of the antitetanus toxoid Ab response analyzed on postimmunization day 56 in ELISA that determined the quantity of IgG1, IgG2a, IgG2b, or IgG3 present. Data are plotted as log\(_{10}\) reciprocal titers ± SEM (n = 3 +/- and 4 −/−). The ages of mice studied ranged from 9 to 16 wk.
6-mo-old, or 1-yr-old animals. T cell-dependent B cell Ig responses were intact with normal serum Ig levels as well as Ab responses to tetanus toxoid. Although NK cell functional activity was normal, Ag-specific CTL activity was decreased in CD7-deficient mice.

Previous work (6, 18) in humans has suggested that CD7 is involved in NK cell proliferation, adhesion, and cytokine production. However, in CD7-deficient mice, NK cell function was normal as assessed by the ability to lyse YAC target cells. While our present work does not directly address NK cell adhesion and cytokine production, our work does imply that CD7 is not necessary for development of NK cells or NK cell lytic activity in mice. Future work focusing on inducible adhesion molecule function and cytokine production in CD7-deficient NK cells is warranted.

Numerous studies have also suggested that CD7 cross-linking induces T-cell and NK-cell cytokine responses (6, 15, 17). CD7-deficient mice responded normally in splenocyte and thymocyte proliferation assays; however, IFN-γ production was decreased in tetanus toxoid-immunized CD7-deficient splenocytes after re-stimulation in vitro. Interestingly, CD7-deficient mouse splenocytes also generated significantly less OVA-specific splenocyte CTL activity than did control mouse splenocytes. Taken together, these results suggested a role for mouse CD7 in the process of generating Ag-specific CTL effector functions in vivo. Given the normal IFN-γ responses in CD7-deficient bulk splenocytes to PHA, ConA, CD3, and OVA peptide, the significance of decreased IFN-γ production following tetanus toxoid stimulation at present is not known. CD3-stimulated IFN-γ was decreased (p = 0.06), while production of supernatant IL-4, IL-10, and TNF-α in all settings tested were normal. Future studies to assess correlates to these observations, such as studies in T cell clones and in vivo responses to various pathogens such as Herpes simplex, Leishmania donovani, and/or Listeria monocytogenes, will directly define any functional roles of CD7 in protective host T cell immune responses.

CD7-deficient mice demonstrated transient increases in thymocyte numbers in 3-mo-old mice, suggesting a possible role for CD7

**FIGURE 4.** Functional responses of CD7-deficient splenocytes and thymocytes to mitogenic and antigenic stimulation. A, Proliferative responses of control and CD7-deficient splenocytes in response to stimulation by Con A, PHA, CD3, CD3 + CD28, and tetanus toxoid. n = 4 +/+ and 4 −/− per experiment. Different groups of animals were used for mitogen-stimulated T cell activation experiments and for tetanus toxoid-stimulated cultures. These data are representative of two experiments performed. B, Proliferative responses of control and CD7-deficient thymocytes in response to stimulation by Con A, PHA, and CD3 + 5 ng/ml rIL-2 (n = 4 +/+ and 4 −/− per experiment). These data are representative of two experiments performed. C, IFN-γ in pg/ml in culture supernatants from splenocytes stimulated in vitro as labeled. n = 8 −/− and 8 +/+ (mitogen-stimulated supernatants) and 6 −/− and 7 +/+ (tetanus toxoid-stimulated supernatants). * Signifies p < 0.02. The age of mice studied was 3 mo.

**FIGURE 5.** NK cell lytic activity in CD7-deficient mice. NK cell activity in poly(I:C)-primed control and CD7-deficient 4-mo-old male mice splenocytes was determined using 51Cr-loaded YAC target cells in a 51Cr-release assay. Shown is the percentage specific 51Cr-release at varying E:T ratios (n = 4 +/+ and 4 −/−). Lytic activity in both CD7−/− and control 1+/1 mice was approximately 1 lytic unit in each. The age of mice studied was 4 mo.
in regulation of murine thymopoiesis. This transient rise in the DP
CD4⁺, CD8⁺ cortical thymocyte population in CD7-deficient mice could
either be due to a partial defect in thymocyte apoptosis or to subtle
effects either on positive or negative thymocyte selection.

CD7 shares many similarities with CD28; both are Ig superfamily
molecules expressed on T cells (1, 4, 40), both signal through
PI-3 kinase (12, 13, 37–39), ligation of both is a comitogenic acti-
vation signal for T cells in concert with TCR triggering (14, 15,
42, 43), and molecule cross-linking leads to T cell cytokine pro-
duction (15, 17, 44, 45) as well as modulation of T-cell adhesion
(16). Unlike CD7, which has no known ligands, CD28 has two
known ligands, B7.1 and B7.2 on APCs and other cell types, that
serve to trigger CD28 in vivo, thus providing key signals for Ag-
driven T cell activation (reviewed in Ref. 46). Given the important
role that CD28-mediated T cell triggering plays in normal APC-T
cell interactions, it was surprising that CD28 knockout mice had
only the phenotype of ineffective Ag-driven B cell Ig isotype
switching and decreased T cell triggering to mitogens and Ags,
with no abnormalities seen in T cell-positive or -negative selec-
tion (41). Given the similarities of CD7 and CD28, it will be of interest
to determine whether double deficiencies of CD28 and CD7 in
mice reveal profound or synergistic deficiencies of T cell activa-
tion; these studies are ongoing.

It is important to note that characterization of thymopoiesis in
BCL-XL transgenic mice demonstrated overproduction of thy-
ocytcs seen at 10 wk of age but not in younger mice. In contrast
to CD7-deficient mice with increases in only DP thymocytes, BCL-X
transgenic mice had increases in triple negative, DP, and single-
positive thymocyte subsets (47). It will be of interest to determine
BCL-X and BCL-2 protein levels in CD7-deficient mice to deter-
mine the roles of these apoptosis-regulatory proteins on thymocyte
survival.

Finally, it is important to point out that with more than 20 yr of
mAb production by many investigators and after 5 yr of repeated
attempts by this laboratory, there has yet to be a mAb produced
that reacts with mouse CD7. The reason for this lack of mCD7
mAbs is unknown, but it may relate to the fact that mCD7, unlike
human CD7, does not have an Ig “hinge,” i.e., a homologous re-
gion proximal to the single Ig C-region domain, and therefore, if
mCD7 is a surface molecule, the mCD7 Ig domain would be pre-
dicted to be located proximally to the T cell lipid bilayer. Thus,
it is important to emphasize that the effects of CD7 deficiency on
thymocyte maturation and CTL activity seen in CD7-deficient
mice conceivably be due to intracellular effects of CD7 de-

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FIGURE 6. CTL activity in CD7-deficient mice. Ag-primed splenocytes. A.
Percentage specific lysis of 51Cr-labeled EG7ova target cells by ovalbumin
peptide-primed splenocytes from CD7-deficient and C57BL/6 control mice. Background
killing of control EL-4 targets not expressing OVA is also shown. B. Data in A
expressed in lytic units (n = 6 −/− and 6 +/+; * signifies p < 0.03, and ** signifies p < 0.01).
The age of mice studied was 3 mo.


