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Immunology

Induction of cytotoxic T lymphocytes by intramuscular immunization with plasmid DNA is facilitated by bone marrow-derived cells

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ABSTRACT **Striated muscle is the predominant site of gene expression after i.m. immunization of plasmid DNA, but it is not clear if myocytes or professional antigen-presenting cells (APCs) of hematopoietic origin present the encoded antigens to class I major histocompatibility complex (MHC)-restricted cytotoxic T lymphocytes (CTL). To address this issue, CTL responses were assessed in mice engrafted with immune systems that were partially MHC matched with antigen-producing muscle cells. Spleen cells (sc) from immunocompetent F_1 H-2^{bxd} mice were infused into H-2^b or H-2^d mice carrying the severe combined immunodeficiency (*scid*) mutation, creating $F_{1sc} \rightarrow$ H-2^b and $F_{1sc} \rightarrow$ H-2^d chimeras, respectively. Immunization with DNA plasmids encoding the herpes simplex virus gB or the human immunodeficiency virus gp120 glycoproteins elicited antiviral CTL activity. $F_{1sc} \rightarrow$ H-2^d chimeras responded to an H-2^d-restricted gp120 epitope but not an H-2^b-restricted gB epitope, whereas $F_{1sc} \rightarrow$ H-2^b chimeras responded to the H-2^b but not the H-2^d-restricted epitope. This pattern of epitope recognition by the sc chimeras indicated that APCs of recipient (*scid*) origin were involved in initiation of CTL responses. Significantly, CTL responses against epitopes presented by the mismatched donor class I molecules were elicited if F_1 bone marrow cells and sc were transferred into scid recipients before or several days to weeks after DNA immunization. Thus, bone marrow-derived APCs are sufficient for class I MHC presentation of viral antigens after i.m. immunization with plasmid DNA. Expression of plasmid DNA by these APCs is probably not a requirement for CTL priming. Instead, they appear to present proteins synthesized by other host cells.**

Immunization with plasmid DNA encoding microbial antigens has provided protective immunity in some animal models and is therefore considered a potentially useful vaccine strategy (1–4). Antigens produced in host cells after intramuscular or intradermal DNA immunization elicit humoral and cellular immune responses (1, 5, 6), including class I major histocompatibility (MHC)-restricted cytotoxic T lymphocytes (CTL) thought to be important for control of intracellular parasites and tumors. Mechanisms of antigen processing and class I MHC presentation to $CD8^+$ T lymphocytes in the immunized animals are poorly understood, in part because the range of cell types that express plasmid DNA *in vivo* has not been fully defined. Gene expression is readily detectable in myocytes and myoblasts after i.m. delivery of plasmid DNA-based expression constructs (7–9). Class I MHC molecules present on the surface of these cells (10) are probably sensitized with epitopic peptides that can engage the T-cell antigen receptor (TcR). Whether this is sufficient to elicit a CTL response is uncertain because only antigen-presenting cells (APCs) of hematopoietic origin express costimulatory molecules that deliver additional T cell activation signals (for review, see ref. 11). On the other hand, uptake and expression of plasmid DNA by macrophages and dendritic cells have not been demonstrated and therefore it is not clear if antigens have direct access to the class I MHC processing pathway of these specialized APCs (12). Macrophages and dendritic cells may also have a scavenger pathway for class I MHC processing of proteins released from damaged cells (13), and it has been proposed that this could facilitate priming of CTL responses by plasmid DNA immunization even if antigen expression is restricted to myocytes (14). Cross-priming experiments that involve immunization of mice with H-2 mismatched cells provide strong support for the existence of this alternate pathway. CTL activity against tumor, H-Y, or minor alloantigens expressed by these histoincompatible cells was restricted by class I H-2 molecules of the recipient animal, suggesting that APCs of host origin participate in priming of the response (15).

In this study, we used a similar cross-priming strategy to assess APC requirements for induction of CTL responses by i.m. immunization of plasmid DNA. Lack of functional B and T lymphocytes in mice carrying the severe combined immunodeficiency (*scid*) mutation facilitates engraftment with allogeneic mononuclear cells from immunocompetent animals (16, 17). Thus, priming of $CD8^+$ CTL responses can be assessed when the adoptively transferred immune system is H-2 matched or mismatched with antigen-producing muscle cells of the *scid* recipient. Immunization with DNA plasmids encoding human immunodeficiency virus type-1 (HIV-1) or herpes simplex virus type-2 (HSV-2) antigens did result in virus-specific CTL responses in *scid* mice reconstituted with spleen cells from immunocompetent donors. However, this required class I H-2 matching between donor lymphocytes and the recipient mouse, suggesting that APCs from the *scid* recipient were required for initiation of CTL activity. Significantly, transfer of immunocompetent spleen and bone marrow cells before or even after DNA immunization resulted in virus-specific CTL activity without any requirement for class I H-2 matching with the *scid* recipient. These results suggest that class I MHC-restricted CTL responses are initiated by bone marrow-derived APCs, possibly by processing viral proteins released from other tissues such as striated muscle.

METHODS AND MATERIALS

Animals. Four- to six-week-old C57BL/6J-scid/SzJ (H-2^b) and C.B-17 (H-2^d) *scid/scid* (*scid*) mice were purchased from The Jackson Laboratory and Taconic Farms, respectively.

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Abbreviations: APC, antigen-presenting cell; bmc, bone marrow cells; CTL, cytotoxic T lymphocyte; HSV-2, herpes simplex virus type-2; MHC, major histocompatibility complex; rVV, recombinant vaccinia virus; *scid*, severe combined immunodeficiency; sc, spleen cells; TcR, T-cell antigen receptor.

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BALB/c (H-2^d), C57BL/6 (H-2^b), and CB6F1 (H-2^{bxd}) mice from Charles River Breeding Laboratories were used between 5 and 8 weeks of age.

DNA Plasmids and Peptides. DNA plasmids and epitopic peptides used in this study are summarized in Table 1. Plasmid pCMV6a120 encodes amino acids 31 (glutamic acid) to 509 (arginine) of the HIV-1 (strain SF2, HIV-1_{SF2}) envelope glycoprotein gp120 as a fusion protein with the human tissue plasminogen activator signal peptide (18). Expression is controlled by the human cytomegalovirus (strain Towne) enhancer/promoter with intron A (18). Expression of amino acids 23–718 of the HSV-2 (strain 333) gB glycoprotein from plasmid pHS216 (designated pCMVgB) is also regulated by these human cytomegalovirus sequences. Plasmid pCMVp55gagpre expresses the entire p55 protein of HIV-1_{SF2}, which includes the p24 core. The hepatitis B virus pre element is included to overcome HIV-1 rev dependence of p55 gag expression.

Synthetic epitopic peptides from HIV-1_{SF2} gp120 [gp120(312–320); aa 312-IGPGRAFHT-320], p24 gag [p24(199–207); aa 199-AMQMLKETI-207], and HSV-2 gB [gB(496–503); aa 496-SSIEFARL-503] were synthesized with free amine N termini and free acid C termini using fluorenylmethoxycarbonyl (Fmoc) solid phase methods by Research Genetics (Huntsville, AL).

Cell Transfers and Immunizations. Splenocytes (5×10^7) and bone marrow cells (bmc) (1×10^7) from 6- to 8-week-old CB6F1 mice were suspended in RPMI 1640 culture medium (GIBCO) containing 10% heat inactivated fetal calf serum and then transferred i.v. to C.B-17 or C57BL/6J-scid/SzJ *scid* mice. One day later, plasmid DNA (100 μg) in 50 μl of phosphate-buffered saline was injected into the tibialis anterior muscles and in some experiments mice were boosted 4 weeks later. Four to six weeks after the last immunization, spleens were removed for CTL assays. In some experiments mice were infected i.p. with 1×10^7 plaque-forming units of recombinant vaccinia viruses expressing the HSV-2 gB (rVVgB) or HIV-1 gag and polymerase (rVVgag/pol) proteins, and spleen cells were removed 4 to 6 weeks later for assay of CTL activity.

Lymphocyte Cultures. Spleen cells (sc) from immunized mice were cultured in 24-well dishes at 5×10^6 cells per well. Of those cells, 1×10^6 were sensitized with synthetic epitopic peptides from HIV-1_{SF2} or HSV-2 proteins at a concentration of 10 μM for 1 hr at 37°C, washed, and cocultured with the remaining 4×10^6 untreated sc in 2 ml of culture medium [50% RPMI 1640 and 50% alpha-MEM (GIBCO)] supplemented with 10% heat-inactivated fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, antibiotics, and 5% interleukin 2 (Rat T-Stim, Collaborative Biomedical Products, Bedford, MA). Cells were fed with 1 ml of fresh culture medium on days 3 and 5, and cytotoxic activity was assessed on day 6.

Cytotoxic Cell Assay. SVBALB (H-2^d) and MC57 (H-2^b) target cells used in the ^{51}Cr release assays express class I but not class II MHC molecules. Approximately 1×10^6 target cells were incubated in 200 μl of medium containing 50 μCi (1 Ci = 37 GBq) of ^{51}Cr and synthetic HIV-1 or HSV-2 peptides (1 μM) for 60 min and washed 3 times. Effector (E) cells were cultured with 5×10^3 target (T) cells at various E/T ratios in 200 μl of culture medium in 96-well round-bottom tissue culture plates for 4 hr. The average cpm from duplicate wells was used to calculate percent specific ^{51}Cr release as described (19).

ELISA Assays. HIV-1 gp120 and HSV-2 gB antibody titers in the serum of immunized mice were measured using ELISA assays as described (20). Serial twofold dilutions of serum were tested for binding to recombinant Chinese hamster ovary cell-derived gp120 or gB glycoproteins, starting at a dilution of 1/25. Titers are reported as reciprocal of the serum dilution that gave a half-maximum optical density reading.

Table 1. DNA plasmids and viral epitopes

Plasmid	Virus gene	CTL epitope	H-2 restriction
pCMV6a120SF2	HIV-1_{SF2} gp120	gp120 (312–320)	H-2D^d
pCMVp55gagPRE	HIV-1_{SF2} core	p24 (199–207)	H-2K^d
pCMVgB	HSV-2 gB	gB (496–503)	H-2K^b

RESULTS

Cellular requirements for priming class I MHC-restricted CTL responses were investigated using DNA plasmids encoding HSV-2 or HIV-1_{SF2} proteins. DNA vectors expressing the viral genes and location of class I MHC-restricted epitopes are shown in Table 1. Intramuscular immunization of C57BL/6 (H-2^b) mice with pCMVgB induced a vigorous CTL response against gB(496–503) (Fig. 1*A*), an octameric H-2K^b-restricted epitope located in the gB protein of HSV-2 (21). Class I MHC-restricted responses were also observed in BALB/c (H-2^d) mice after immunization with the pCMV6a120 or pCMVp55 gagpre DNA plasmids, which encode the HIV-1_{SF2} gp120 envelope and p55 gag proteins, respectively. Responses elicited by pCMVp55gagpre were directed against p24(199–207), an H-2K^d restricted epitope in the HIV-I_{SF2} p24 protein (22) (Fig. 1*B*), whereas pCMV6a120 induced strong cytolytic activity against gp120(312–320), an epitope in the V3 domain of gp120 (19, 23) (Fig. 1*C*).

Immunodeficient mice carrying the *scid* mutation can be engrafted with allogeneic mononuclear cells (16, 17), and thus this animal model was used to assess the role of professional APCs in induction of CTL responses after i.m. injection with

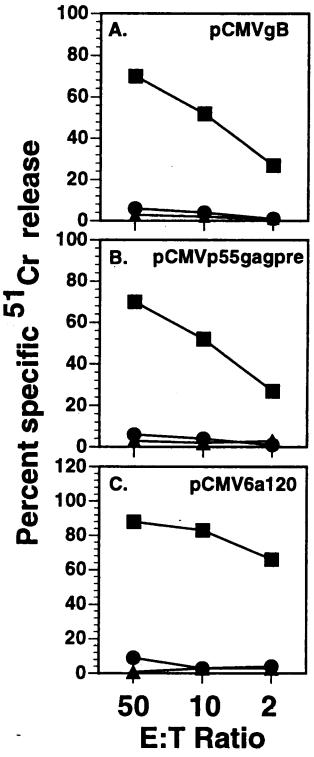

FIG. 1. CTL responses in plasmid DNA-immunized mice. (*A*) pCMVgB-immunized C57BL/6 effectors tested against untreated (●) or gB(496–503)-sensitized (■) MC57 targets, or gB(496–503)-sensitized SVBALB targets (▲). (*B*) pCMVp55gagpre-immunized BALB/c effectors tested against untreated SVBALB (●), p24(199–207)-treated SVBALB (■), and p24(199–207)-treated MC57 (▲) targets. (*C*) pCMV6a120-immunized BALB/c effectors tested against untreated SVBALB (●), gp120(312–320)-treated SVBALB (■), and gp120(312–320)-treated MC57 targets (▲). Results shown are from individual mice and are representative of 10 to 20 replicates.

the DNA plasmids. C.B-17 (H-2^d) *scid* mice were infused intravenously with 5×10^7 sc from normal CB6F1 (H-2^{bxd}) mice, and the resulting $F_{1sc} \rightarrow$ H-2^d chimeras were challenged with recombinant viruses expressing either HSV-2 gB or HIV-1 p24 gag proteins to ensure successful engraftment. Infection of the sc chimeras with rVVgag/pol elicited CTL activity against SVBALB (H-2^d) target cells pulsed with the p24(199–207) peptide (Fig. 2*A*). Levels of killing were equivalent to those generated in normal BALB/c mice (Fig. 2*A*). Infection of the $F_{1sc} \rightarrow$ H-2^d chimeras with rVVgB also resulted in H-2^b-restricted cytolytic activity against gB(496–503)-sensitized MC57 target cells (Fig. 2*B*). These results demonstrate that semiallogeneic (H-2^{bxd}) sc are functional in the chimeric mice, and that virus infection can induce CTL responses against epitopes presented by class I MHC molecules that are either matched (H-2^d) or mismatched (H-2^b) with the C.B-17 *scid* host.

To determine if plasmid DNA encoding viral antigens could induce similar immune responses, H-2^d *scid* mice were immunized in the tibialis anterior muscles with 100 μg of either pCMVgB or pCMV6a120 at weeks 0 and 4. At week 8, humoral and cellular immune responses were assessed. Unreconstituted H-2^d *scid* mice immunized with pCMV6a120 generated neither antibodies (Table 2) nor CTL activity (Fig. 3*A*) against gp120. Immunization of $F_{1sc} \rightarrow$ H-2^d spleen cell chimeras with this plasmid did elicit gp120-specific antibodies (Table 2) and H-2^d-restricted CTL activity against the gp120(312–320) epitope (Fig. 3*B*). Significantly, an H-2^b-restricted CTL response against the gB(496–503) epitope of HSV-2 was not detected in the $F_{1sc} \rightarrow$ H-2^d chimeric mice after pCMVgB-immunization (Fig. 3*C*) even though they clearly made antibodies against the recombinant gB protein (Table 2). To determine if this unresponsiveness resulted from a lack of T cells specific for the gB(496–503) epitope, pCMVgB-immunized $F_{1sc} \rightarrow$ H-2^d chimeras were infected at week 8 with rVVgB. As expected from the results shown in Fig. 2*B*, CTL activity specific for the H-2K^b-restricted gB(496–503) epitope was detected 4 weeks later (Fig. 3*D*), demonstrating that immunocompetent H-2^b-restricted T lymphocytes were still present.

Induction of H-2^d but not H-2^b-restricted CTL responses by i.m. immunization of the $F_{1sc} \rightarrow$ H-2^d chimeras with plasmid DNA suggested a role for recipient cells in antigen presentation. This could involve either antigen-producing myocytes or professional APCs that develop normally from bone marrow precursors of *scid* mice (23, 24). To investigate the latter possibility, C57BL/6J-scid/SzJ (H-2^b) *scid* mice were reconstituted with 5×10^7 F_1(H-2^{bxd}) sc alone or in combination with 1×10^7 bmc. $F_{1sc} \rightarrow$ H-2^b chimeras immunized with pCMVgB had a strong gB-specific CTL response (Fig. 4*A*),

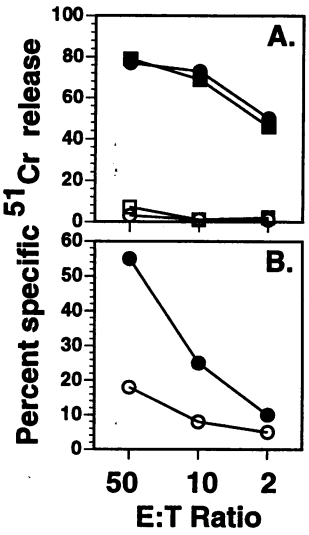

FIG. 2. Virus-induced CTL responses in sc reconstituted *scid* mice. (*A*) Effectors from VVgag/pol-infected BALB/c mice (circles) or $F_{1sc} \rightarrow$ H-2^d chimeras (squares) were tested against untreated (○, □) or p24(199–207)-treated (●, ■) SVBALB targets. (*B*) $F_{1sc} \rightarrow$ H-2^d chimeras infected with rVVgB were assessed for lytic activity against untreated (○) or gB(496–503)-sensitized (●) MC57 targets. Groups of three to four infected mice were tested, and representative results from one individual are shown.

again demonstrating that this activity can be generated when donor sc share H-2 compatibility with the recipient. CTL responses against the HSV-2 epitope were of similar magnitude in $F_{1sc+bmc} \rightarrow$ H-2^b mice reconstituted with sc and bmc (Fig. 4*B*). CTL priming across the histocompatibility barrier was again not observed in *scid* mice receiving sc alone; in this case, pCMV6a120-immunized $F_{1sc} \rightarrow$ H-2^b chimeras did not mount a CTL response against the H-2^d-restricted gp120(312–320) epitope (Fig. 4*C*) even though they had serum antibodies against the gp120 protein (data not shown). Significantly, when these H-2^b mice were infused with F_1 sc and bmc, gp120-specific CTL activity was readily detected (Fig. 4*D*). Identical results were obtained in the reciprocal $F_{1sc+bmc} \rightarrow$ H-2^d chimeras, where H-2^b and H-2^d-restricted CTL activity was induced by immunization with the pCMVgB and pCMV6a120 plasmids, respectively (data not shown). These results indicate that MHC matching between antigen-expressing myocytes and responder T cells is not a requirement for CTL priming. Indeed, the results suggest that after i.m. delivery of plasmid DNA, cells derived from the bone marrow are involved in class I MHC presentation of encoded antigens.

Table 2. Antibody responses in plasmid DNA-immunized C.B-17 (H-2^d) *scid* mice

			Antibody titers against antigen			
			gB		gp120	
DNA plasmid*	Animal	H-2^{bxd}, sc†	Week 0	Week 8	Week 0	Week 8
pCMV6a120	BL-027	–	<25	<25	<25	<25
	BL-030	–	<25	<25	<25	<25
	BL-033	+	<25	<25	<25	2384
	BL-034	+	<25	<25	<25	1360
pCMVgB	BL-021	+	<25	3328	<25	<25
	BL-023	+	<25	2568	<25	<25
	BL-024	+	<25	6969	<25	<25
	BL-025	+	<25	5340	<25	<25

*The indicated DNA plasmid (100 μg) was injected into the tibialis anterior muscle at weeks 0 and 4. Sera collected from individual animals at weeks 0 and 8 were assessed for gB and gp120-specific antibodies as described.

†Sc (5×10^7) from CB6F1 (H-2^{bxd}) mice were injected intravenously into C.B-17 mice 24 h before DNA immunization.

Viral proteins produced in professional APCs after uptake of plasmid DNA would have direct access to the class I MHC processing pathway. Alternatively, it is possible that these APCs have mechanisms for processing exogenous proteins released from other cell types. To distinguish between these possibilities, H-2^d *scid* mice received F_1 sc and bmc 1 day before or 8 and 21 days after immunization with the pCMVgB plasmid. As expected, transfer of F_1 sc and bmc 1 day before immunization with the plasmid resulted in strong CTL activity against the H-2K^b-restricted gB(496–503) epitope in the H-2^d scid host (Fig. 5*A*). Significantly, responses of equal magnitude were detected even when the F_1 sc and bmc were transferred at 8 (Fig. 5*B*) or 21 (Fig. 5*C*) days after immunization with the pCMVgB DNA plasmid.

DISCUSSION

Cellular requirements for priming virus-specific humoral and T cell-mediated immune responses by i.m. injection of plasmid DNA were investigated in H-2^b or H-2^d *scid* mice reconstituted with semiallogeneic CB6F1 (H-2^{bxd}) mononuclear cells. *Scid* mice receiving sc from F_1(H-2^{bxd}) donors had antibodies against the HIV-1 and HSV-2 envelope glycoproteins, indicating that mature B and T lymphocytes from secondary lymphoid organs were sufficient for transfer of humoral immunity. Splenocytes also reconstituted CTL activity against the HSV-2 and HIV-1 antigens but recognition of specific epitopes was governed by the parental H-2 haplotype of the recipient mouse. To summarize, F_{1sc} → H-2^d chimeras responded to the H-2D^d restricted epitope in HIV-1 gp120 but not the H-2K^b restricted epitope in HSV-2 gB, whereas F_{1sc} → H-2^b chimeras had the opposite pattern of responsiveness. This requirement for H-2 compatibility indicated that APCs from the *scid* recipient were involved in class I MHC presentation of epitopic peptides to splenic CD8$^+$ T cells from the CB6F1 donor. Antigen-producing myocytes are not necessarily the only candidate for this role, as specialized APCs develop normally in scid mice and are capable of initiating immune responses (24, 25). Indeed, professional APCs of hematopoietic origin do appear to be involved because administration of sc and bmc from CB6F1 (H-2^{bxd}) donors reconstituted CTL activity against H-2^b- and H-2^d-restricted epitopes regardless of the class I H-2 haplotype of the *scid* recipient. Professional APCs are present in secondary lymphoid tissue such as spleen, and yet only bone marrow cells could transfer APC function to the *scid* mice. Few mature dendritic cells are found in the bone marrow (26) but there are large numbers of precursors that give rise to macrophages and dendritic cells (27–29). Stem cells

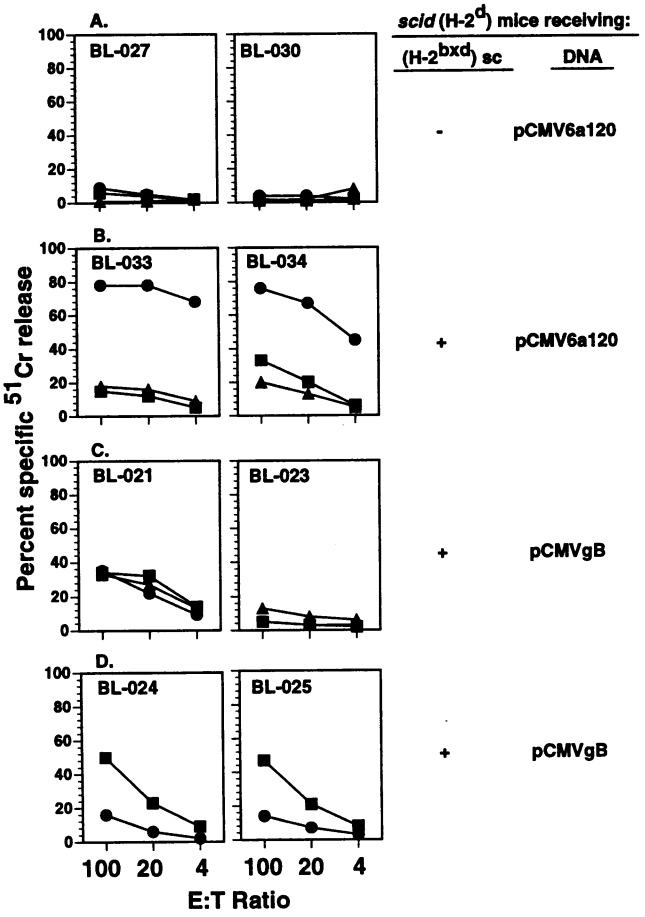

FIG. 3. CTL responses in H-2^d *scid* mice immunized with plasmid DNA. C.B-17 (H-2^d) *scid* mice that were (*A*) untreated or (*B*) reconstituted with 1×10^7 CB6F1 (H-2^{bxd}) sc were immunized with pCMV6a120 at weeks 0 and 4. CTL activity was assessed at 8 weeks against untreated SVBALB (▲), or gp120(312–320)-treated SVBALB (●) and MC57 (■) targets. (*C*) Effectors from pCMVgB-immunized F_{1sc} → H-2^d chimeras were tested against untreated MC57 (●) or gB(496–503)-sensitized MC57 (■) and SVBALB (▲) targets. (*D*) F_{1sc} → H-2^d) sc mice immunized at weeks 0 and 4 with pCMVgB were infected at week 8 with rVVgB. CTL activity was measured 4 weeks later against untreated (●) or gB(496–503)-treated (■) MC57 targets. Groups of four mice were tested for CTL activity, and representative results from two animals are shown.

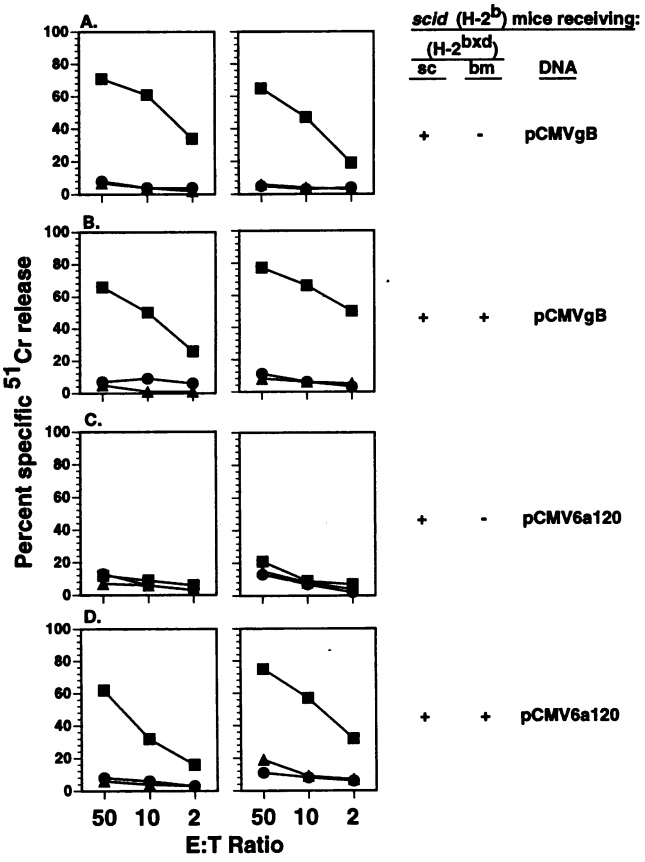

FIG. 4. CTL responses in H-2^b *scid* mice immunized with plasmid DNA. F_1 → H-2^b chimeras that received (*A*) sc or (*B*) sc and bmc from CB6F1 mice were immunized once with pCMVgB and CTL activity was measured 4 weeks later against untreated MC57 (●), gB(496–503)-sensitized MC57 (■), and gB(496–503)-sensitized SVBALB (▲) targets. F_1 → H-2^b chimeras receiving (*C*) sc or (*D*) sc and bmc were immunized with pCMV6a120 and CTL activity was assessed against untreated SVBALB (●), gp120(312–320)-sensitized SVBALB (■), and gp120(312–320)-treated MC57 (▲) targets. Groups of three to four mice were tested for CTL activity and representative results from two individuals are shown.

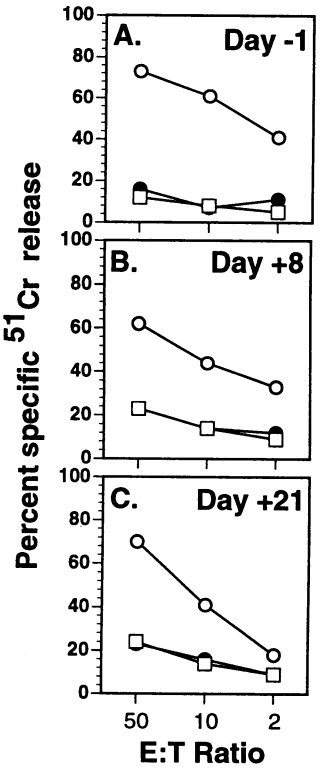

FIG. 5. Effect of immune reconstitution before and after DNA immunization on CTL responses. H-2^d *scid* mice immunized i.m. with the pCMVgB plasmid on day 0 were reconstituted with F_1 (H-2^{bxd}) sc and bmc at day −1 (*A*), day +8 (*B*), or day +21 (*C*). At day 56, spleen cells from three mice per group were pooled, restimulated with the gB(496–503) peptide, and tested for CTL activity against MC57 cells that were untreated (●) or sensitized with the gB(496–503) peptide (○). Control SVBALB cells sensitized with the gB(496–503) peptide were also tested (□). Assay time was 4 hr.

present in primary lymphoid tissue might therefore provide more complete repopulation of the APC compartment than spleen cells. Bone marrow also contains precursor T lymphocytes but their H-2 restriction pattern is imposed by the parental haplotype of the *scid* mouse in which they mature (30). Thus, mature T lymphocytes present in the F_1 spleen or bone marrow are the most likely source of H-2^b- and H-2^d-restricted effector cells in the *scid* recipients.

Two mechanisms could account for sensitization of class I MHC molecules on bone marrow-derived APCs with epitopic HIV-1 or HSV-2 peptides. Antigens synthesized in the cytoplasm of professional APCs would clearly have direct access to the class I MHC processing pathway (for review, see ref. 12). Myocytes are the only cells with detectable levels of gene expression after i.m. immunization with plasmid DNA (7–9), although intradermal administration of DNA resulted in uptake by a variety of cell types, including some with dendritic morphology (31). The possibility that small numbers of specialized APCs were sensitized by endogenously produced antigens cannot be excluded. However, the demonstration that CTL responses were generated even when F_1 sc and bmc were transferred up to 3 weeks after DNA immunization of a histoincompatible *scid* host strongly suggests that professional APCs are not necessarily required to take up and express plasmid DNA. Instead, they appear to present proteins synthesized by cells of the recipient animal, most probably myocytes. The existence of a pathway for class I MHC processing of exogenous antigens is supported by several other studies (15). Induction of CD8$^+$ CTL responses against minor alloantigens, H-Y, or tumor antigens expressed by histoincompatible cells predicted a pathway by which cellular debris gains access to the class I MHC processing machinery of the host APCs (15). In support of this hypothesis, Huang and coworkers (32) demonstrated that influenza nucleoprotein-specific CTLs are efficiently induced by immunization with H-2 mismatched tumor cells expressing this viral protein, and antigen presentation was solely dependent on host APCs that originated in the bone marrow. It is noteworthy that the HSV-2 gB and HIV-1 gp120 proteins are targeted to the secretory pathway, and thus may be the substrate for priming CD8$^+$ CTL responses. On the other hand, antigens released from damaged myocytes may also be involved since cell-associated (33, 34) or aggregated proteins (35) are very efficient immunogens for CTL priming. The identity of bone marrow-derived APCs involved in priming of CTL responses is unknown, but *in vitro* studies indicate that macrophages and dendritic cells can process extracellular antigens for class I MHC presentation (35–39).

Our results are consistent with the view that induction of CTL activity requires specialized APCs that can deliver two activation signals, one mediated by the TcR and the other by accessory molecules such as CD28 (11). Evidence of the importance of costimulatory molecules on APCs has been obtained in transgenic mice, where ectopic synthesis of the CD28 ligand B7.1 in pancreatic islet cells that also expressed a viral glycoprotein was necessary to provoke a virus-specific CTL response (40). Transfer of somatic cells to lymph nodes may circumvent the requirement for coexpression of class I MHC and costimulatory molecules on the same APCs (41), but it seems unlikely that this applies to myocytes tranfected *in situ* by plasmid DNA immunization. Our results do not necessarily rule out a role for striated muscle in presentation of antigen to CD8$^+$ T cells; human myoblasts cultured in the presence of cytokines induce *in vitro* proliferation of class II HLA-restricted T cells, and thus they are considered facultative APCs that amplify immune responses initiated by professional APCs (42). The animal model described here should be useful for defining the contribution of professional and nonprofessional APCs to induction of CTL responses by plasmid DNA immunization, and evaluating strategies for augmenting their function by inclusion of genes for cytokines or costimulatory molecules.

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