Brief Definitive Report

THYMIC SELECTION OF H-2-INCOMPATIBLE BONE MARROW CELLS IN SCID MICE

Differences in T Help for Induction of B Cell IgG Responses Versus Cytotoxic T Cells

By R. M. ZINKERNAGEL, E. RÜEDI, A. ALTHAGE, H. HENGARTNER, AND G. REIMANN

From the Institut of Pathology, University Hospital, CH-8091 Zürich, Switzerland; and the Institute of Medical Microbiology, University of Ulm, Federal Republic of Germany

The question of how precursor T cells mature in the thymus and how their restriction specificity for self-major histocompatibility gene products is selected for and functions in vivo particularly in histoincompatible combinations, is still somewhat controversial (1–3). The recently discovered murine model of immunodeficiency may offer new possibilities to analyze this question. Mice (C.B-17 H-2^d) with a homozygous genetic defect on chromosome 2 cannot generate either TCRs or Igs; they suffer from a severe combined immunodeficiency disease (SCID) (4, 5). This immunodeficiency can readily be reversed by the transplantation of H-2-compatible stem cells from normal heterozygous littermates or of H-2-compatible normal stem cells. Because of their profound immunodeficiency, SCID mice readily accept H-2-incompatible stem cell grafts without prior irradiation; therefore, this model offers the possibility to study T lymphocyte interactions in an H-2^d host with an H-2^d thymus, with functional T cells and B cells exclusively of H-2^b origin in the presence of APC of both H-2^d and H-2^b type. We found that such SCID chimaeras generated excellent virus-specific cytotoxic T cells of H-2^b origin that were exclusively restricted to host H-2^d; in contrast, these mice failed to generate T help-dependent IgG responses to neutralizing virus determinants.

Materials and Methods

Mice. *nu/nu* BALB/c A BOM and *nu/nu* C57BL/6 BOM mice were obtained from Bomholtgard, Ltd., Ry, Denmark. C.B-17 SCID mice were bred under standard pathogen-free conditions in the animal colony of the University of Ulm. Breeding pairs of C.B-17 SCID mice were a generous gift from Dr. R. A. Phillips (Toronto, Canada). All mice were maintained under specific pathogen-free (SPF) conditions and used at 10–12 wk of age. Immunocompetent control mice were C57BL/6 and BALB/c (H-2^d), and were obtained from the Institut für Zuchthygiene (H-2^b), Tierspital, Zürich.

Chimeras. Bone marrow cells from nude mice were injected intravenously into sex- and age-matched C.B-17 SCID mice (2×10^7 cells/mouse). Reconstituted SCID mice and control mice were maintained 3–6 mo under SPF; they were transferred to conventional housing facilities and tested 2–8 wk later.

Virus. The vaccinia virus (Lancy isolate) was obtained from the Schweizerisches Impfinstitut, Bern (2); the lymphocytic choriomeningitis virus (LCMV) WE (2, 6) and vesicular stomatitis virus (VSV) (7) have been described in detail; cytotoxic T cell activities were

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measured 6 d (vaccinia) or 8 d (LCMV) after infection. VSV was UV inactivated by exposure to a germicidal lamp (Philips Electronic Instruments, Inc., Mahwah, NJ, 15 W, 7 G) for 5 min at a distance of 10 cm.

Secondary Stimulation and Alloreactive Mixed Lymphocyte Cultures In Vitro. Spleen cells from mice primed with vaccinia virus (4×10^6 per 16-mm well) were stimulated with H-2-compatible spleen cells (2×10^6/well) infected with UV-inactivated virus or left uninfected for allostimulation (2, 6). Spleen cells were infected with vaccinia virus at a multiplicity of ~1:1 (with respect to original pfu before UV inactivation) for 2 h at 37°C and then washed twice before being irradiated and added to responder cells. The medium used was Iscove's modification of Dulbeccos' medium supplemented with penicillin and streptomycin, 5% heat-inactivated FCS, and 5×10^{-5} M 2-ME. Cells were added in a total volume of 2 ml/well and cultured for 5 d. Four identical wells of the various cultures were pooled, washed, and resuspended in 1.5 ml. 100 µl of this pool or a 1:3 or 1:9 dilution was then tested against the standard 10^4 target cells. The highest concentration corresponded to 1.6×10^6 spleen cells before culture to one target cell.

Cytotoxicity Assay. The target cells and the procedures used have been described in detail previously (2, 6).

Anti-H-2 Treatment and H-2 Typing. Standard numbers of spleen cells (usually 2×10^7) were treated twice with the following antisera or mAbs plus Low-Tox^R rabbit complement diluted 1:12–1:15. K7309.15 (anti-H-$2K^b$) was used as undiluted culture supernatant at 300 µl per 1–2 × 10^7 cells; a serum pool of hyperimmunized (C3H × BALB/c) (H-2^k × H-2^d)F_1 anti-BALB/b (H-2^b) and a serum pool (C3H × C57BL/10) (H-2^k × H-$2k^b$)F_1 anti-BALB/c (H-2^d) were used at 50–100 µl of undiluted serum per 2×10^7 spleen cells in a total volume of 300–500 µl of balanced salt solution. Viability was assessed by trypan blue exclusion. After treatment, effector cells were all adjusted to the same concentration of viable cells to yield the indicated E/T ratios in the assay.

Neutralizing Anti-VSV Antibody Titers. A standard neutralization assay was used as described elsewhere (7). Titers assessed on day 4 reflect IgM antibodies only; sera from day 12 were pretreated with 0.1 M 2-ME to yield IgG titers.

Results

SCID mice did not show a measurable CTL response after infection with vaccinia virus (Table I). Their NK cell activity against YAC-1 cells, however, was comparable with that of control mice, as has been previously described (8). If reconstituted with syngeneic BALB/c (H-2^d) or allogeneic C57BL/6 (H-2^b) stem cells 9–12 wk previously, SCID mice responded excellently to vaccinia virus and developed CTL responses comparable with immunocompetent control mice. Cytotoxic activity in allogeneically (H-2^b) reconstituted SCID mice was exclusively mediated by donor H-2^b (Tables I and II) cells, which were restricted exclusively to recipient H-2^d. The H-2 treatment of effector cells revealed that roughly half of the spleen cells of these allogeneically reconstituted SCID mice were of donor and the other half of recipient origin (Table I). These experiments were repeated with two independent batches of reconstituted SCID mice in five independent experiments using vaccinia virus or LCMV (data not shown) with identical results. However, whereas control SCID mice failed to mount a T-independent neutralizing IgM or a T cell–dependent IgG response to VSV (Fig. 1), syngeneically H-2^d-reconsitituted SCID mice generated neutralizing IgM and IgG titers comparable with that of control mice. Allogeneically H-2^b-reconstituted SCID mice mounted a normal T-independent IgM response, but no measurable IgG response to VSV (Fig. 1).

Restimulation experiments in vitro with virus primed SCID + H-2^b lymphocytes and infected H-2^b stimulator cells failed to reveal H-2^b-restricted effector T

TABLE I

Antiviral Cytotoxic T Cell Activities in SCID (H-2^{d}) Mice Reconstituted with H-2^{d} (BALB/c nu/nu) or H-2^{b} (C57BL/6 nu/nu) Bone Marrow Cells

Mouse number	Chimeras or controls + reconstituting stem cells	E/T ratios	Percent-specific ^{51}Cr release from target cells				
			MC57G(H-2^{b})		(D2 H-2^{d})		
			Vaccinia	Uninfected	Vaccinia	Uninfected	YAC-1
1	SCID BALB/c control (H-2^{d})	70:1	<1	<1	<1	<1	17
		23:1	<1	<1	<1	<1	4
		8:1	<1	<1	<1	<1	4
2	SCID + BALB/c *nu/nu* (H-2^{d})	70:1	<1	<1	66	<1	9
		23:1	<1	<1	32	<1	<1
		8:1	<1	<1	12	<1	<1
3	SCID + C57BL/6 *nu/nu* (H-2^{b})	70:1	2	<1	45	<1	18
		23:1	<1	<1	27	<1	6
		8:1	<1	<1	10	<1	4
4	BALB/c (H-2^{d})	70:1	<1	<1	71	8	30
		23:1	<1	<1	72	1	18
		8:1	<1	<1	40	4	7
5	C57BL/6 (H-2^{b})	70:1	82	1	14	7	33
		23:1	57	2	6	3	14
		8:1	25	<1	1	2	7
Spontaneous release (5 h)			14	19	19	22	23
			Percent-specific ^{51}Cr release from Vaccinia D2 (H-2^{d})				
			Anti-H-2^{d} + C		Anti-H-2^{b} + C	C only	None
3	SCID + C57BL/6 *nu/nu* (60% H-2^{d}, 40% H-2^{b})	70:1	61		3	74	61
		23:1	46		2	24	44
		8:1	24		3	18	25
4 + 5	BALB/c (H-2^{d}) + C57BL/6 (H-2^{b})	70:1	2		98	100	98
		23:1	7		71	68	55
		8:1	6		43	39	33

Mice were infected with 10^{7} pfu of vaccinia virus 6 d before the test.

cells (data not shown). This further strengthened the conclusion that T cell specificity for thymic H-2 was strict. The exclusive restriction of primary virus-specific CTL to recipient H-2 type may not be explained by exclusive H-2^{d}-specific stimulation of effector cells in the periphery for the following reasons. Vaccinia virus (Lancy) is mainly expressed in mononuclear phagocytic cells (6, 9), and about half of the mononuclear cells in the periphery including antigen presenting cells (see Table II) are of donor origin. This is documented by the finding that spleen cells from H-2^{b}-reconstituted SCID mice stimulated both H-2^{b}- and H-2^{d}-specific alloresponses equally efficiently; they were also able to function as APCs for virus-primed H-2^{b} responder cells (Table II).

Discussion

The presented results show that primary anti-viral CTL responses in allogeneically (H-2^{b}) reconstituted SCID (H-2^{d}) mice were excellent, whereas, T help–dependent primary IgG anti-VSV responses were not generated. The following not

TABLE II
Antigen Presentation and Allostimulation by SCID + H-2^b Spleen Cells

Responder spleen cells	Stimulator spleen cells	Dilution of effector cells	Percent-specific release MC57G (H-2^b) Vaccinia	Uninfected
C57BL/6 (H-2^b) Vaccinia virus primed	C57BL/6 Vaccinia	1	55	10
		⅓	41	4
		⅑	23	3
C57BL/6 (H-2^b) Vaccinia virus primed	C57BL/6 uninfected	1	8	12
		⅓	10	7
C57BL/6 (H-2^b) Vaccinia virus primed	SCID + H-2^b Vaccinia	1	50	6
		⅓	32	4
		⅑	13	2
Spontaneous release (4.5 h)			15	20
			P815 (H-2^d)	MC57G (H-2^b)
C57BL/6 (H-2^b)	SCID + H-2^b	1	94	9
		⅓	93	4
		⅑	74	3
BALB/c (H-2^d)	SCID + H-2^b	1	16	97
		⅓	9	100
		⅑	6	64
Spontaneous release (4.5 h)			10	20

For details for cultures and test see Materials and Methods. Comparable results were obtained with lymphocytes from an additional chimera.

mutually exclusive explanations may apply. The data support the notion that T help may not be necessary for the induction of CTL responses as has been concluded from experiments using depletion of L3T4⁺ (CD4⁺) helper T cells in vivo or in vitro (10, 11). The presented experiments cannot prove or disprove the necessity of T help for the generation of CTL as has been discussed repeatedly (9-13). They may, however, illustrate that soluble mediators suffice for induction, and that no direct physical (H-2 restricted) contact between T helper cells and CTL is necessary. This view will be discussed in greater detail below and is compatible by the apparent absence of class II MHC antigens on cytotoxic T cells (14). Although suppressive mechanisms may be postulated to explain the finding, we have no evidence for them; in view of the normal IgM response of B cells and the normal cytotoxic T cell re-

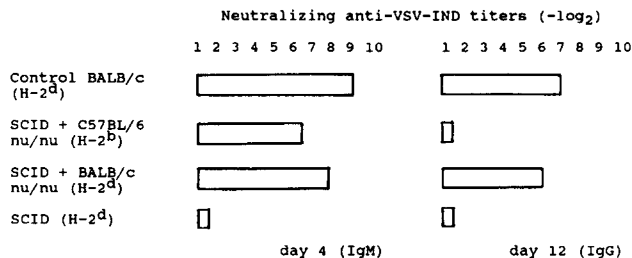

FIGURE 1. T-independent IgM and T-dependent IgG anti-VSV-IND neutralizing antibody responses against 2 × 10^7 UV-inactivated VSV-IND; mean titers of four sera, SEM were all smaller than one dilution step of two.

sponses, hypothetical suppression of T help for IgG producing B cells would have to be very selective.

The data suggest that T helper cells must interact directly with B cells in an MHC-restricted fashion to deliver help for IgG production in vivo. Since antigen presentation in the periphery appears to be about equally represented by both H-2^d and H-2^b cells (see Table II), T help expressed by H-2^b T cells specific for VSV plus H-2^d should be able to recognize VSV-antigenic determinants plus H-2^d on APCs of host origin and could induce lymphokine release. This possible pathway is apparently not sufficient to induce B cells but might suffice to induce CTL responses. In contrast, the same T cells cannot recognize VSV determinants on H-2^b B cells of donor type, because they are restricted solely to H-2^d. The absence of an anti-VSV IgG response indicates therefore a mandatory MHC-restricted contact between T helper cells and B cells in vivo for IgG production. The differences in T help requirements observed probably signal more stringent regulatory needs to maintain tolerance to self expressed by B as compared with CTL cells (15) to limit the danger of autoimmunity.

Summary

Mice with congenital severe combined immunodeficiency disease (SCID) failed to mount either a T cell-independent IgM or T cell-dependent IgG anti-vesicular stomatitis virus (VSV) Indiana (IND) response. They did not generate cytotoxic T cells against lymphocytic choriomeningitis virus (LCMV) or vaccinia virus, but exhibited NK cell-like activities. When SCID mice were given bone marrow from syngeneic BALB/c (H-2^d) *nu/nu* mice, all immune responses were expressed at control levels. If SCID mice were reconstituted with allogeneic H-2^b C57BL/6 *nu/nu* bone marrow, the following primary anti-viral immune responses were measured. T-independent IgM anti-VSV-IND were normal, but T-dependent IgG anti-VSV-IND responses were absent. Cytotoxic T cell responses to LCMV and vaccinia virus were within normal ranges, were donor cell mediated, and were specific exclusively for the recipient SCID H-2^d type. Since antigen presentation by spleen cells was functional in these chimaeras, the presented results indicate that (*a*) thymic selection of T cell restriction is strict; and (*b*) the type of T help necessary for B cells depends upon H-2-restricted contact between T and B cells, whereas, such contact-dependent help is not mandatory for the induction of virus-specific cytotoxic T cells.

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