MAJOR HISTOCOMPATIBILITY COMPLEX RESTRICTION
OF SOLUBLE HELPER MOLECULES
IN T CELL RESPONSES TO ALTERED SELF*

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Molecular products of the major histocompatibility genetic complex (MHC)[1] function in pathways involving cell-cell interactions during immune responses. Histocompatibility for the classical MHC antigens, such as the H-2K and H-2D,L products in the mouse, is generally required for cytotoxic effector functions relative to altered self modes of responses. Such histocompatible limitations placed on antigenic recognition are referred to as MHC restriction (1–3). Cellular interactions that require compatibility for antigens encoded by the H-2I region occur during the initial and regulatory phases of the immune response. Also during this period, T cells elaborate soluble factors. Soluble T cell factor preparations, in general, appear not to be MHC restricted (4). Most cases in which MHC restriction has been observed, the soluble factors apparently exert their effects on T cells. The MHC restriction of a soluble, carrier-specific, enhancing T cell factor described by Tada et al. (5) was found to be associated with the H-2IA region and required Thy-1-bearing cells for its effect. Nonspecific factors that suppress allogeneic T cell proliferative responses as described by Rich et al. (6) require homology at the H-2IC region. A strict requirement for I-J-region compatibility between antigen-specific suppressor factor and acceptor cells has also been shown in a keyhole limpet hemocyanin-dinitrophenyl plaque-forming cell system by Tada et al. (5); again the soluble molecules apparently bind to T cells. Strict I-J restrictions, however, have not been extensively observed in suppressor systems studied by others (4). Soluble helper molecules in allogeneic test systems also do not reflect MHC restriction (7). Exceptions can be found, however, when soluble help is produced between two strains that differ by a limited segment of the MHC ([8]; and J. M. D. Plate, C. A. McDaniel, L. Flaherty, J. H. Stimpfling, R. W. Melvold, and N. Q. Martin, manuscript in preparation) and with appropriate assay conditions. It is my contention that the detection of MHC-restricted soluble mediators of immune differentiation can be obscured in assay systems that use techniques in vitro, especially when Ia$^+$ T cells (I-A region encoded) are present. The demonstration of I-J-region-associated restriction of allogeneically derived soluble help for B cells by Delovitch and Sohn (8) was dependent upon the removal of T cells. Further cell surface phenotype analyses were not undertaken in that study. We would suggest, on

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the basis of our findings, that Ia^+ T cells can readily be triggered by soluble helper molecules to either produce other nonspecific lymphokines such as T cell growth factor (TCGF) (Interleukin-2 [IL-2]) (9) or to alter physically the MHC-incompatible message in such a way that it can successfully be transmitted to its collaborating H-2-compatible counterparts (prekiller cells, cohelper cells, B cells and/or suppressor subpopulations). Furthermore, MHC restriction of soluble help in T cell effector systems may apply only to particular T cell subpopulations. We have observed MHC restriction of soluble helper molecules in the absence of I-A-region-bearing Ia^+ T cells only in syngeneic responses which involve largely Lyt-$1,2,3,^+$ prekiller cells, and not in allogeneic responses.

Materials and Methods

Mice. The B10 congenic lines B10.D2, C57BL/10, and B10.A(5R) as well as DBA/2 female mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Breeding pairs of B10.A(4R) and B10.A(18R) were obtained from Dr. J. Stimpfling, The McLaughlin Research Institute, Great Falls, Mont.; the lines were maintained locally by strict brother and sister mating. The D2.GD and A.SW lines were obtained from Dr. R. Melvold and Dr. N. Ponzio at Northwestern University Medical Center, Chicago, Ill.; the B10.LG mice were generously provided by Dr. C. David, Mayo Clinic and Medical School, Rochester, Minn.

Antiserum. The (A × B10.A)F₁ antiserum to B10.A(5R) was provided by the resources branch of National Institute for Allergy and Infectious Diseases, Bethesda, Md. This antiserum contains antibodies to an Ia determinant encoded by the I-A region and that is expressed on regulatory T cells (10). We have termed this Ia determinant as Ha (10).

Antiserum Treatments. B10.D2 strain lymph node cells at a concentration of 1.0×10^7 or $1.5 \times 10^7/0.5$ ml were treated for 15 min at 37°C with normal mouse serum (NMS) or (A × B10.A)F₁ anti-B10.A(5R) serum (anti-Ia) diluted 1:5 with RPMI-1640 medium. An equal volume of appropriately diluted rabbit serum was added as a source of complement, and the cells were incubated for an additional 30 min in a 37°C shaking water bath. The cells were washed three times, and counts of viable cells were made.

Sensitization In Vitro. Responding cells ($5 \times 10^6/$ml) were mixed with an equal or suboptimal number of syngeneic spleen cells that had been modified by trinitrobenzenesulfonic acid (TNP modified) (11, 12) and irradiated with 2,000 rad from a ^{137}Cs source. The addition of a suboptimal number of stimulators permits us to evaluate amplification effects of helper factor supernates (HFS) on either untreated or NMS-treated cells. The cell mixtures were pelleted by centrifugation at 1,200 rpm for 10 min and resuspended in complete medium supplemented with 20% HFS. The cell mixtures were then transferred to microplates such that 5–10 replicate wells each contained 0.2 ml cell suspension in either RPMI-1640 or Dulbecco's modified minimum essential medium (Grand Island Biological Co., Grand Island, N. Y.) that contained 10% human serum and 5 mM Hepes buffer plus 50 µg L-arginine, 75 µg L-glutamine, 100 U penicillin G, and 75 µg kanamycin sulfate per ml. After incubation 4 or 5 d at 37°C in a humidified atmosphere of 5% CO_2 the replicate cultures were pooled, washed, counted and assayed for killer cell activity.

TNP Modification. Nucleated spleen cells that had been irradiated or P815-X2 mastocytoma cells and/or concanavalin (Con-A) blast cells previously labelled with ^{51}Cr were conjugated with 2,4,6-trinitrobenzenesulfonic acid (TNBS) according to published protocols (11, 12). Cells were washed with 10 mM phosphate-buffered saline, pH 7.3, and incubated in this buffer plus 10 mM TNBS acid. The spleen cells were then washed in RPMI-1640 medium that contained 10% human serum and were prepared for culture. The TNP-conjugated P815-X2 mastocytoma cells and/or Con-A blast cells were washed in RPMI-1640 plus 10% calf serum and utilized as targets for the killer-cell assay in microplates according to established protocols (7).

HFS. All supernates were derived from two-step tissue culture procedures as described in detail previously (7). Responding strain lymph node cells that had been activated for 3–5 d with allogeneic or TNP-altered syngeneic spleen cells were washed and recultured with fresh nonirradiated stimulating strain cells. After a 6-h incubation period, the supernates which then

contained helper factor activity were harvested and filtered through 0.22-μm filters to remove cellular debris. The various HFS utilized in experiments reported in this manuscript include the following: (a) B10.D2 plus C57BL/10 (2,000 rad); (b) C57BL/10 plus B10.D2 (2,000 rad); (c) B10.D2 plus B10.D2-TNP (2,000 rad); (d) C57BL/10 plus C57BL/10-TNP (2,000 rad); (e) B10.A(5R) plus A.SW (2,000 rad); (f) B10.A(18R) plus A.SW (2,000 rad); (g) C57BL/10 plus A.SW (2,000 rad); (h) A.SW plus B10.A(5R) (2,000 rad); (i) D2.GD plus DBA/2J (2,000 rad); (j) B10.A(4R) plus B10.D2 (2,000 rad); and (k) B10.LG plus B10.A (2,000 rad).

Results

Ia-bearing Immunoregulatory T Cells. At least two different types of Ia-bearing cells are essential for the generation of altered-self cytolytic effector cells. Glass-adherent Ia$^+$ accessory cells that do not require H-2 homology with responding T cells for their effectiveness have been described by Pettinelli et al. (13) as resistant to irradiation and to lysis by antiserum to the Thy-1 antigen. We have demonstrated that Ia-bearing T cells serve immunoregulatory functions in the generation of syngeneic TNP-directed effector cells (10). Furthermore, these immunoregulatory Ia$^+$ T cells do not adhere to nylon wool, and apparently must be radiosensitive, as their functions are not performed after replacement by the irradiated stimulator cells (Fig. 1). B10.D2 cells that had passed through nylon wool columns (14) and had been treated with
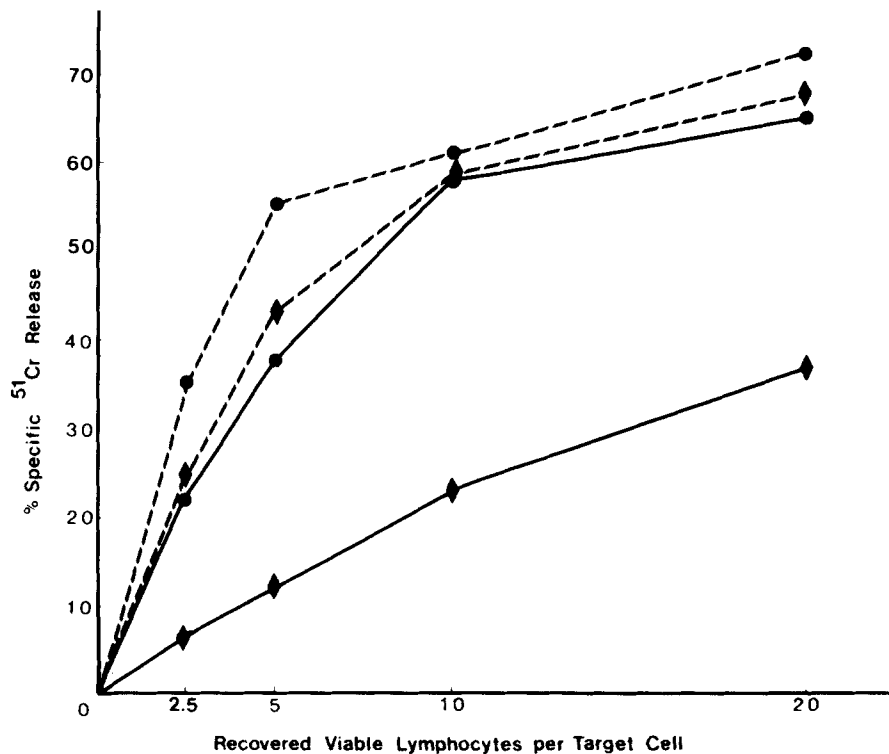

FIG. 1. Effect of anti-Ia treatments and HFS on nylon wool-purified T cells. B10.D2 lymph node cells (LNC) (1.5 × 10^7/0.5 ml) that had been passed over nylon wool fibers were treated with (●) NMS or (◆) anti-Ha serum [(A × B10.A)F$_1$ anti-B10.A(5R)] plus complement. The remaining viable cells were reconstituted to 5 × 10^6 cells/ml and mixed with an equal number of irradiated TNP-modified B10.D2 spleen cells, then cultured at 0.2 ml/well with (—) medium or (---) medium supplemented with 20% HFS produced by B10.D2 cells stimulated with C57BL/10 (2,000 rad).

anti-Ia-containing antiserum plus complement exhibited reduced capacities to generate killer cells specific for syngeneic TNP-altered cells. HFS from allogeneic cell mixtures (B10.D2 plus irradiated C57BL/10 cells) did, however, support the generation of killer cells in these B10.D2 cultures.

Evidence for MHC Restriction of Help.   Soluble helper activities produced by syngeneic B10.D2 cells and by allogeneic C57BL/10 cells were effective in NMS-treated cell cultures (Fig. 2). Little killer cell activity was observed without addition of HFS (data not included). Evidence for MHC restriction of soluble help was observed after removal of Ia^+ responding cells. Thus, HFS from C57BL/10 cells that had been stimulated with B10.D2 irradiated cells, could not support the generation of B10.D2 syngeneic TNP-specific killer cells in the cultures of cells treated with antiserum to Ia (Fig. 2 A). HFS from syngeneic responder cells (B10.D2 stimulated with irradiated C57BL/10 cells) enabled the B10.D2 cells remaining after anti-Ia lysis to generate syngeneic TNP killer cells (Fig. 2 B). We have not found soluble help to be similarly restricted in allogeneic systems (7).

Active HFS can also be obtained from cells that respond to syngeneic TNP-altered cells (Fig. 3). After removal of the Ia^+ cells from the B10.D2 responding strain, the syngeneically produced HFS also exhibited MHC restriction of helper activity (Fig. 4). Thus, HFS produced by B10.D2 plus irradiated B10.D2-TNP stimulating cells and by C57BL/10 plus irradiated C57BL/10-TNP cells both provided help to amplify the responses of NMS-treated B10.D2 cells, but only the syngeneic B10.D2 HFS could furnish help for the B10.D2 cells pretreated with anti-Ia serum.
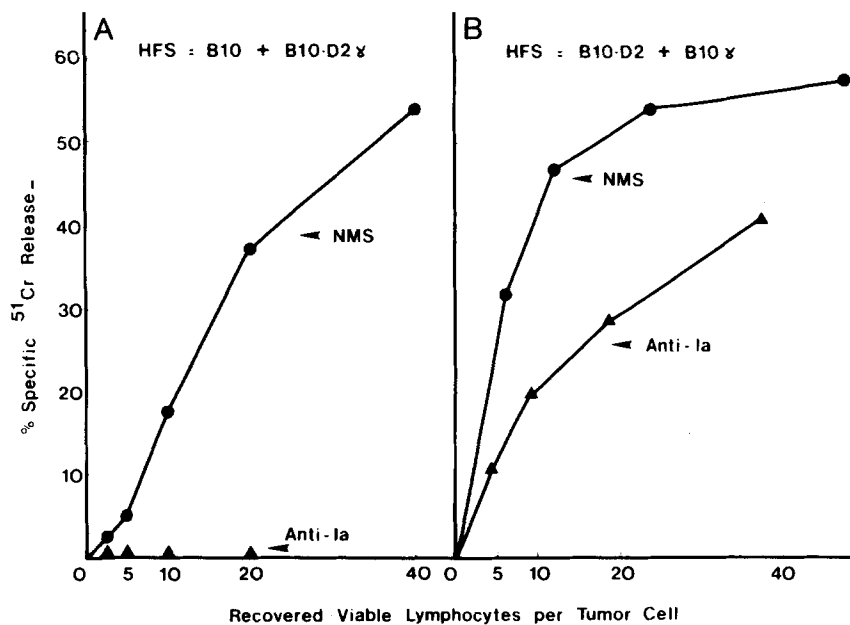

Fig. 2.   Evidence for MHC restriction of soluble help revealed after anti-Ia serum lysis of responding cell cultures. B10.D2 LNC were pretreated with (●) NMS or (▲) (A × B10.A)F₁ anti-B10.A(5R) serum plus complement and cultured in medium with a suboptimal of irradiated TNP-modified B10.D2 spleen cells. HFS derived from either (A) C57BL/10 plus B10.D2 (2,000 rad) (B10 + B10.D2γ) or (B) B10.D2 plus C57BL/10 (2,000 rad) (B10.D2 + B10γ) cultures were added at a 20% final concentration.
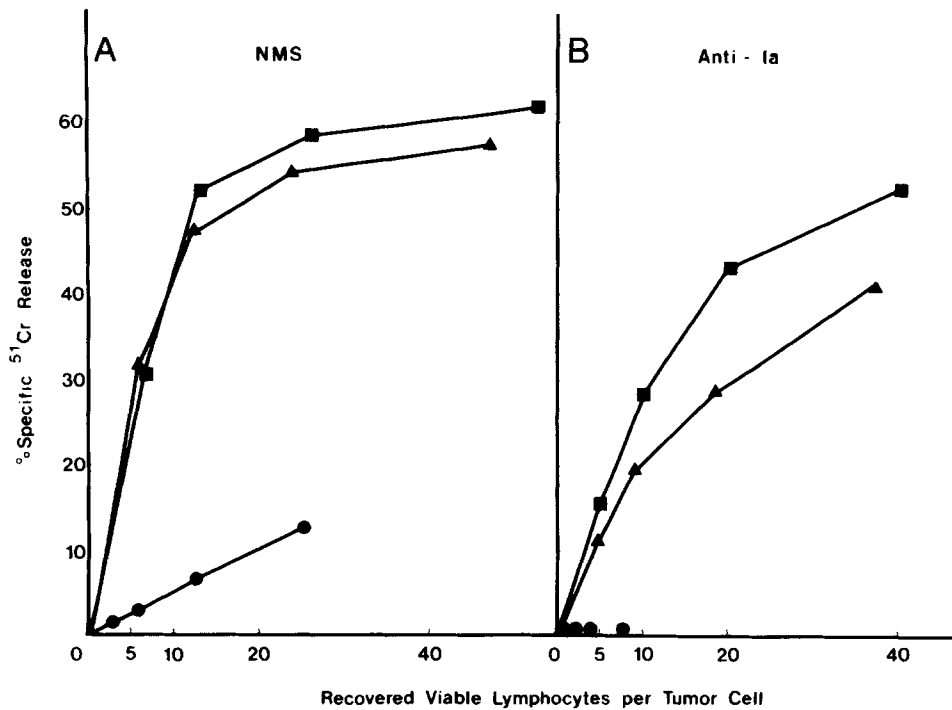
Fig. 3.   Effective soluble help produced in response to TNP-altered self. B10.D2 LNC (1.5 × 10^7/ 0.5 ml) were treated with (A) NMS or (B) (A × B10.A)F$_1$ anti-B10.A(5R) serum (Anti-Ia). The remaining viable cells were cultured with an equal number of TNP-modified and irradiated B10.D2 spleen cells in (●) medium, or in medium supplemented with 20% HFS derived from (■) B10.D2 + B10.D2-TNP (2,000 rad) and (▲) B10.D2 + C57BL/10 (2,000 rad) culture procedures (7).
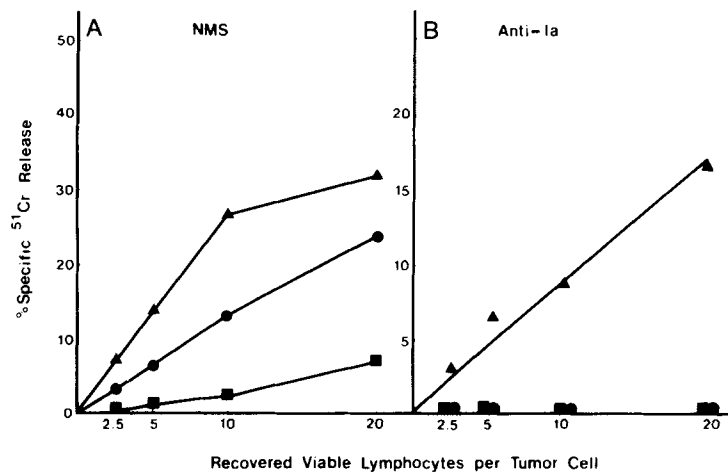

Fig. 4.   MHC restriction of soluble help produced in response to TNP-altered self. Viable B10.D2 LNC remaining after lysis with (A) NMS or (B) (A × B10.A)F$_1$ anti-B10.A(5R) serum (Anti-Ia) were cultured with irradiated TNP-modified B10.D2 spleen cells in medium supplemented with 20% HFS derived from (▲) B10.D2 + C57BL/10 (2,000 rad), (●) C57BL/10 + C57BL/10-TNP (2,000 rad) and (■) C57BL/10 + B10.D2 (2,000 rad) culture procedures (7). No killing was observed by cells cultured in unsupplemented medium.

Genetic Mapping of the Region Responsible for MHC Restriction of Soluble Help. The MHC genetic region responsible for the restriction of soluble help has been mapped to I-A through the use of supernates produced by a number of recombinant and congenic lines of mice (Table I). To assess helper factor activity in these various supernatants, B10.D2 strain cells were treated with either anti-Ia-containing serum or NMS plus complement. The remaining cells were set up in culture with syngeneic TNP-coupled cells. Supernates derived from the 5R, 4R, 18R, and D2.GD recombinant lines all augmented the responses of the cells pretreated with NMS (Figs. 5 and 6). The 5R, 18R, and 4R HFS, however, could not support the generation of syngeneic TNP-effector cells in those cultures pretreated with anti-Ia serum (Figs. 5 and 6).

TABLE I

H-2 Haplotypes Used to Map Restriction for Soluble Help *

Strain	Haplotype	H-2 regions						
		H-2K	H-2I				H2-S	H-2D
			A	B	J	E/C		
B10.D2	H-2^d	d	d	d	d	d/d	d	d
C57BL10	H-2^b	b	b	b	b	b/b	b	b
B10.A(18R)	H-2^{i18}	b	b	b	b	b/b	b	d
B10.A(5R)	H-2^{i5}	b	b	b	k	k/d	d	d
D2.GD	H-2^{g2}	d	d/b‡	b	b	b/b	b	b
B10.LG	H-2^{df}	d	f	f	f	f/f	f	f
B10.A(4R)	H-2^{h4}	k	k	b	b	b/b	b	b
A.SW	H-2^s	s	s	s	s	s/s	s	s

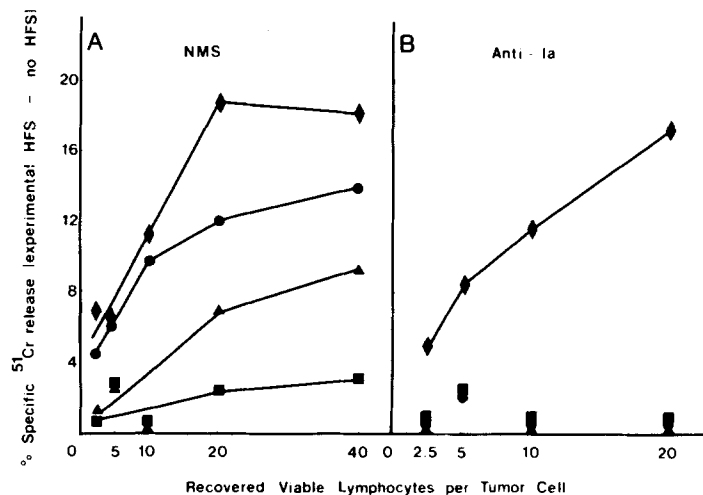* Taken from Klein et al. (15).
‡ Modification from Jones (16).

FIG. 5. Genetic mapping of H-2 regions responsible for MHC restriction of soluble help. B10.D2 LNC (10⁷/0.5 ml) were treated with (A) NMS and (B) (A × B10.A)F₁ anti-B10.A(5R) serum (Anti-Ia). The viable cells were then set up with syngeneic irradiated and TNP-modified B10.D2 spleen cells in culture medium supplemented with 20% HFS produced by (◆) D2.GD + DBA/2 (2,000 rad), (●) C57BL/10 + B10.D2 (2,000 rad), (▲) B10.A(4R) + B10.D2 (2,000 rad), and (■) B10.LG + B10.A (2,000 rad) culture procedures (7).
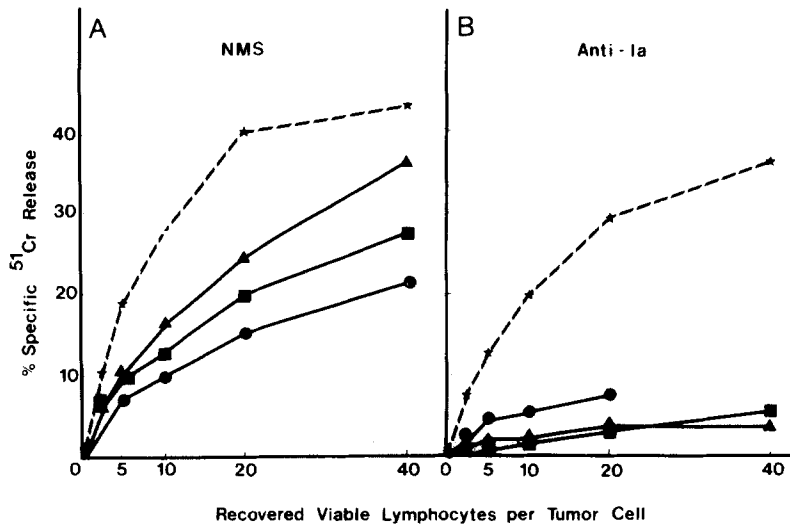
Fig. 6.   Genetic mapping of H-2 regions responsible for MHC restriction of soluble help. Viable B10.D2 LNC after treatment with (A) NMS or (B) (A × B10.A)F₁ anti-B10.A(5R) serum (Anti-Ia) were cultured with irradiated and TNP-modified B10.D2 spleen cells in medium supplemented with 20% HFS derived from (▲) B10.A(5R) + A.SW (2,000 rad), (■) B10.A(18R) + A.SW (2,000 rad), (●) C57BL/10 + A.SW (2,000 rad), and (★) A.SW + B10.A(5R) (2,000 rad) culture procedures (7). No killing was observed by cells cultured in unsupplemented medium.
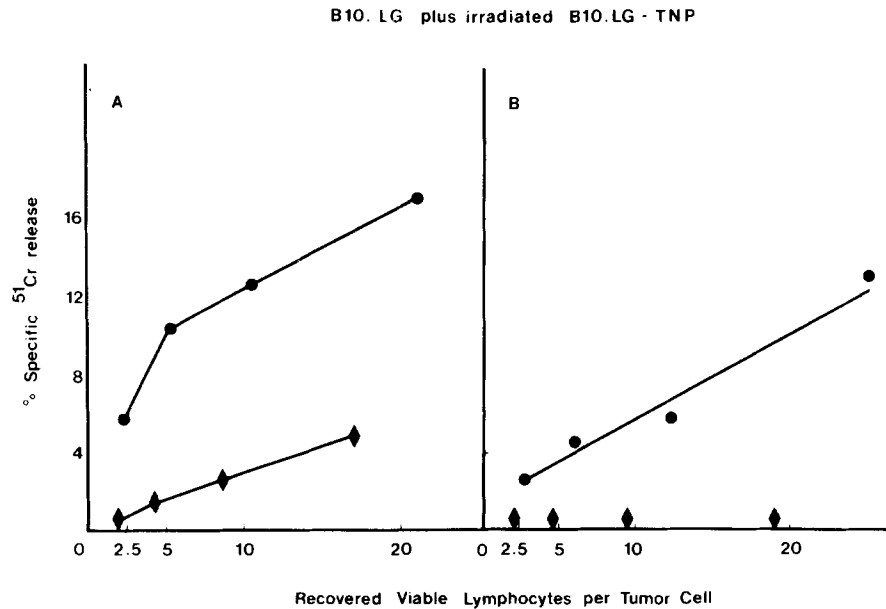

Fig. 7.   HFS produced by B10.LG cells contain active help for B10.LG syngeneic cells. B10.LG LNC were cultured with (A) an equal number (10⁶/well) or (B) a suboptimal number (2.5 × 10⁵) of irradiated and TNP-modified B10.LG spleen cells in (◆) medium and (●) medium supplemented with 20% B10.LG + B10.A HFS.

HFS produced by either D2.GD against its congenic partner, DBA/2, (Fig. 5), or A.SW against an H-2- and mls-incompatible strain, B10.A(5R) (Fig. 6), were capable of supporting the generation of syngeneic killer cells in these cultures.

Genetic mapping of the MHC locus (or loci) responsible for the restriction of soluble help was accomplished as follows. The H-2^d allele provides support, while the H-2^b allele does not. The H2-D^d region is eliminated as a possible restricting locus (or loci) with the 18R recombinant (Table I). The 5R line confirms this conclusion and, furthermore eliminates the I-C/E and H-2S regions. Active soluble help provided by the D2.GD strain demonstrates that restricting elements are encoded to the left of I-B, as this recombinant expresses H-2K, and a portion of I-A region of the H-2^d haplotype. The B10.LG recombinant shares only the H-2K^d region with the H-2^d haplotype and does not contribute active help to the cells treated with anti-Ia serum. MHC restriction of soluble help, therefore, can be mapped within the genetic region located to the right of H-2K and left of I-B, or to what is presently known as the H-2IA region. The B10.A(4R) confirms the H-2-compatibility requirement for soluble help to mediate the generation of syngeneic killer cells in the absence of Ia$^+$ T-cells. Help provided by the A.SW strain suggests that mls loci stimulate the production of nonspecific factors that are not MHC restricted. This possibility is being investigated further. MHC restriction relative to mls stimuli also has not been observed in other systems (17, 18).

The low level of help provided to the NMS-treated cells by the 4R and, particularly, the B10.LG strain, indicates further that H-2I-region compatibility between responder strains and helper factor producer cells is required for optimal help, even in the presence of Ia-bearing T cells. The HFS produced by B10.LG cells are active, as they do indeed provide help for their syngeneic responders to their own TNP-altered cells (Fig. 7). The low level of help afforded to B10.D2 cells by HFS derived from the B10.LG strain cells was not due to the induction of suppressor cells (data not shown).

Discussion

There apparently is a large diversity of cellular interactions and biochemical pathways that leads to the effector stage of an immune response and its control. Such diversity has been recognized on a cellular level for some time, but it is just becoming appreciated at the molecular-immunobiological level. The complexities of cellular subtypes and molecular communicants are best illustrated in suppressor systems as recently presented and reviewed by Germain and Benacerraf (4). Even this level of understanding has not yet become possible for helper systems in cell-mediated responses largely as a result, I believe, of the effectiveness of nonspecific soluble help in amplifying responses and obscuring those pathways specifically involved in a given response. Nonspecific soluble help may contribute to the expansion of an immune response but probably at a late stage rather than an early initiation stage of that response (J. M. D. Plate et al., manuscript in preparation). The ready production of highly active nonspecific soluble help in vitro may be a result of a polyclonal activation of memory helper cells by Ia molecules on the antigen-presenting cells. The data presented here demonstrate that in the presence of Ia$^+$ T cell subpopulations, nonspecific soluble factors can trigger biochemical pathways such that events associated with either antigen-specific and/or MHC-restricted factors or even other cell subsets can not readily be discerned. Specifically, we have demonstrated MHC-

restriction in the utilization of soluble help by T cells responding to TNP-altered syngeneic cells. These observations were dependent upon the removal of Ia-bearing T cells from the responding cell suspension.

B10.D2 and C57BL/10 strains of mice, although of different H-2 haplotypes, have in common an H-2IA-region encoded cross-reactive Ia molecule that can be detected by an anti-Ia.8 serum (19). This region also codes for an Ia determinant that we have termed Ha and that is expressed on a T cell subpopulation (10). Whether or not Ia.8 is also associated with the Ha molecule is under investigation. These common Ia identities may allow helper molecules produced in response to H-2-associated stimuli to interact with either strain cells as was observed in our cell cultures pretreated with normal mouse serum. Upon lysis of Ia-bearing cells from the B10.D2 TNP-responder population, however, HFS produced by C57BL/10 cells could no longer help the remaining B10.D2 cells to generate TNP-killers. HFS produced by syngeneic cells; i.e., B10.D2; could support the generation of TNP-killers. Through the use of appropriate recombinant and congenic mice we were able to map the MHC region that governs restriction of soluble helper molecules to the I-A region. The I-A region also largely determines the restricting elements demonstrated for T plus antigen-presenting cell interactions (13, 20; reviewed in [17, 21]).

The D2.GD and B10.LG strains contributed significantly to the genetic mapping of the MHC region responsible for determining restriction in the use of soluble help for syngeneic TNP responses. It has recently been suggested that the recombinational event giving rise to the H-2^{g2} haplotype of the D2.GD strain occurred within the gene for A_e (E_β) (16). Hence, all genes to the right of the A_e (E_β) gene could be eliminated from consideration in our mapping studies. These findings, however, do not rule out the intragenic recombinant or mutant A_e (E_β) gene product itself as a possible contributor to MHC restriction of soluble help as the A_e (E_β) molecule was still precipitable with anti-H-2^d antiserum indicating that at least a portion of the molecule maintained the configuration of the H-2^d-encoded gene product (16).

MHC restriction of soluble help has not been evident in allogeneic T cell systems (7) where the largest contribution of prekiller cells is derived from the Lyt-2,3$^+$ memory subpopulation (22, 24). Syngeneic TNP-altered self prekillers are within the Lyt-1,2,3$^+$ subpopulation (23, 24). The inference is that the MHC-restricted soluble factor specifically signals the Lyt-1,2,3$^+$ cells to further differentiate in response to recognition of the altered self stimuli. The helper signal could be delivered directly to the Lyt-1,2,3$^+$ prekiller cells or via a second helper cell subpopulation. A requirement for two distinct types of helper T cells has been suggested for B cell responses (25–27). Only one of these helper T cell subsets, Th2, expresses Ia antigens on its cell surface (25, 26). It has been suggested that Th2 can augment Th1 and B cell interactions via carrier recognition (25–27) and that its signal can be replaced with nonspecific Con-A-induced factors (26). It is of interest to note that in a previous description of an I-A-region-associated restriction of soluble help, T cells were required for the further effective transmission of helper signals to the B cells for antibody production (5). Thus, the discrepancy between the allogeneic versus syngeneic system could be explained by either of two possibilities: (a) that intermediary Th1 T cells function only in syngeneic and not in allogeneic responses or (b) that Lyt-1,2,3$^+$ cells recognize only an MHC-restricted signal whereas Lyt-2,3$^+$ cells can also receive nonrestricted

signals. The cell subtype responsible for MHC restriction of soluble help in the TNP system is under further investigation.

HFS probably contain an array of distinct molecules, some with functions relative to the regulation of immune responses. The nature of the particular function, the cell subtype receiving the helper signal and the biochemical composition of each molecule may have subtle differences. Each assay is complicated by the diversity of cell types and various factors that contribute to consequential steps or alternatives in the pathway. By dissecting out some of the cell subpopulations involved through the use of anti-Ia serum and by using a syngeneic TNP system that probably reflects primary rather than secondary memory responses, we have at least demonstrated that some soluble helper molecules do display regulatory rules established for the intact cells; e.g., MHC restriction of soluble help in T cell responses to altered self.

Summary

Evidence for major histocompatibility complex (MHC) restriction of soluble helper effects was observed in the generation of syngeneic killer T cells to trinitrophenyl-altered self. Ia-bearing T cells obscure the observation of such interactions, thus, must be removed to detect MHC restriction of nonspecific soluble helper factor supernates (HFS). Genetic mapping studies demonstrated that the strain producing HFS must be compatible in the H-2IA region with the strain utilizing the helper molecules for optimal helper signals to be delivered.

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