MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED ANTIGEN PRESENTATION TO ANTIGEN-REACTIVE T CELLS BY B LYMPHOCYTE TUMOR CELLS*

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To generate a humoral T cell-dependent immune response to a soluble protein antigen, at least three distinct classes of immunocompetent cells—antigen presenting cells, T cells, and B cells—must interact in a concerted manner (1–4). During the initial part of this interaction, soluble antigen is thought to be taken up by Ia-positive antigen-presenting cells (APC)[1] (presumably macrophages or dendritic cells) and processed before being presented to antigen-reactive T helper cells. The APC processes the protein antigen in a way that both native and denatured forms of the same protein exhibit a high degree of cross-reactivity to antigen-reactive T helper cells (5). This observation suggests that a protein antigen may be denatured and/or degraded during antigen processing. Antigen-reactive T helper cells are thought to recognize a specific restriction site on the Ia molecules that are expressed by the APC in conjunction with a specific determinant on the processed protein antigen (6–8). Whether the Ia and processed antigen are physically associaed or separate on the APC plasma membrane is still a controversial issue. After recognition, the antigen-reactive T cells become activated T cells that can interact with B cells directly or indirectly through soluble mediators to initiate B cell differentiation and antibody production. Although a considerable amount of data has been published concerning many aspects of antigen presentation, the biochemical events that occur during antigen processing and those that control APC-T_H cell interactions are largely unknown. This is due in great part to an inability to purify sufficient numbers of homogeneous APC.

We describe a series of tumor lines that are capable of presenting soluble protein antigens to antigen-reactive continuous T cell lines in a major histocompatibility complex (MHC)-restricted fashion. These tumor cells express both I-A and I-E subregion Ia antigens and have the phenotypic characteristics of lymphocytes rather than macrophages or dendritic cells. The characterization of the antigen-presenting functions of the tumor cells presented in this report indicate that the tumor cells have

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[1] *Abbreviations used in this paper:* APC, antigen-presenting cell; LPS, lipopolysaccharide; mb, sperm whale myoglobin; MHC, major histocompatibility complex; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

many of the functional antigen-presenting characteristics previously thought to be limited to macrophages. The tumor cells should be a very useful model system for determining the biochemical events that occur in antigen-processing and for determining the potential interactions between processed antigen and Ia molecules on the plasma membrane of these APC.

Materials and Methods

Mice. DBA/2, C57BL/6, and (A/J × C57BL/6)F_1 (B6A) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, or were bred in the Mayo Clinic animal facilities.

Tumor Cells. WEHI 5, WEHI 3, WEHI 231, WEHI 265.1, WEHI 265, and HUCL-1 tumor cells were grown in culture media consisting of Dulbecco's minimum essential medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), 15% fetal calf serum (or 15% horse serum), 3×10^{-5} M 2-mercaptoethanol, 12 mM Hepes, 100 U/ml penicillin and 100 μg/ml streptomycin, and 2×10^{-3} M glutamine. The L10A2J tumor line is a subline of the spontaneous L10A BALB/c lymphoma originally described by Kim et al. (9). The original L10A tumor cells were reported to express both μ and κ immunoglobulin chains. The L10A cells that we obtained express cytoplasmic κ chain but not μ chain (Dr. M. Kuehl, personal communication). The L10A2J subline was selected for enhanced expression of I-A subregion Ia antigens with a radioimmunoassay, using the monoclonal 17/227 (anti-Ia.15) antibody. The tumor cells were grown in culture media consisting of RPMI 1640 (Gibco Laboratories), 10% horse serum, 3×10^{-5} M 2-mercaptoethanol, 12 mM Hepes, 100 U/ml penicillin and 100 μg streptomycin, and 2×10^{-3} M L-glutamine. The T2 and D2N virally induced cell lines (10) were generously provided by Dr. B Chesebro (Rocky Mt. Laboratory, Hamilton, Mont.). The strain of origin and phenotypic characteristics of each of these cell lines are summarized in Table I. A detailed description of the cell surface phenotypic markers of these cell lines and the biochemical characterization of their Ia antigens is described elsewhere.[2]

Antisera. The 17/227 (anti-Ia.15), 116.32 (anti-Ia.19), and 13/18 (anti-Ia.7) monoclonal antibodies (11) are all IgG_{2a} immunoglobulins obtained from Dr. G. Hammerling, Heidelberg, Germany. The monoclonal antibodies were purified on protein A Sepharose (12) and used at

TABLE I

Phenotypic Characteristics of Tumor Cells Tested for Antigen Presentation

Tumor cell*	Strain of origin	Tumor designation	Ia Antigens‡	FcγR§	Phagocytosis‖	Ig¶	α-Napthyl** butyrate esterase	Antigen presentation‡‡
L10A2J	BALB/c	B Lymphoma	++	+	−	k§§	−	+
WEHI 5	(BALB/c × NZB)F_1	B Lymphoma	++	+	−	μ, k	1–2%+	+
T2	BALB/c	Leukemia	+	+	−	−	−	+
WEHI 231	(BALB/c × NZB)F_1	B Lymphoma	+	+	−	μ, k	−	+
D2N	DBA/2	Leukemia	++	+	−	−	−	+
WEHI 265	BALB/c	Monocytic leukemia	±	ND‖‖	ND	−	ND	±
WEHI 265.1	BALB/c	Monocytic leukemia	−	ND	ND	−	+	−
HUCL-1	BALB/c	Monocytic	−	ND	ND	−	ND	−
WEHI 3	BALB/c	Monocytic	−	ND	ND	−	ND	−

* All tumor lines are nonadherent in in vitro culture.

‡ Ia antigens were isolated from lentil lectin purified, [^{3}H]leu Triton X100 solubilized cell extract precipitated with (B10.GD × B6)F_1 anti-B10.HGT (anti-I^d) alloantisera and quantitated on SDS-PAGE (13). Amounts of Ia are relative to amounts isolated from BALB/c splenocytes (++) (see text).

§ EA (sheep erythrocyte) rosette assay (9).

‖ Latex particle (1.1μ) ingestion verified by electron microscopy (9).

¶ Ig cell surface expression determined by fluorescence activated cell sorter (30).

** α-Napthyl butyrate esterase stain done according to Li et al. (31).

‡‡ This paper.

§§ Personal communication, Dr. M. Kuehl, University of Virginia.

‖‖ Not done.

[2] E. Walker, L. L. Lanier, N. L. Warner, and D. J. McKean. Characterization and functional properties of B lymphoma and Mϕ tumor cell lines in accessory cell replacement assays. Manuscript submitted for publication.

a concentration of 1 mg/ml. The (B10.LG × B6)F_1 anti-B10.HTG (anti-I^d), A.TH anti-A.TL, and (B10.LG × C3H/HEJ)F_1 anti-C3H.OL (anti-H-$2D^d$) alloantisera were generously provided by Dr. Chella David, Mayo Clinic.

Antigens. Sperm whale myoglobin (mb) was purchased from Sigma Chemical Co., St. Louis, Mo. and was purified by ion exchange chromatography (13). GAT (lot 10) was purchased from Miles Laboratories, Inc., Elkhart, Ind.

Lipopolysaccharide (LPS) Stimulation. Tumor cells at 5×10^6/ml were stimulated with LPS (14) at 50 μg/ml for 48 h and then washed free of LPS before being added to the T cell proliferation assay.

Soluble Antigen-reactive T Cell Cultures. The antigen-reactive T cells were induced as previously described (15). A detailed report (16) on the establishment and maintenance of mb-reactive T cell lines will be reported elsewhere. The T cells are maintained as long-term continuous cultures by restimulation at 10–14-d intervals with antigen and syngeneic irradiated (3,300 rad) spleen cells. Viable antigen-reactive T cells were recovered 10–14 d after antigenic restimulation and used to assay specific antigen presentation by splenic or tumor APC. In this assay, 10^4 T cells were mixed with 10^6 spleen or 10^5 tumor cells that had been treated with either mitomycin C (10^7 cells/ml treated with 50 μg mitomycin C for 30 min at 37°C) or 3,300 rad of γ-irradiation (^{137}Cs source, Isomedix). Cells were incubated in 0.2 ml RPMI 1640 medium (described above but containing 10% fetal calf serum) containing 1–3 μM antigen in microtiter plates (3040; Falcon Labware, Oxnard Calif.) for 48 h at 37°C. [^{3}H]thymidine (Research Products International Corp., Mt. Prospect, Ill.; 2 μCi/well) was then added to each well, the cells were harvested 16 h later on filter paper, and ^{3}H incorporation was quantitated with standard liquid scintillation counting. All assays were done in triplicate, and the standard deviations of each mean value were within 15%. Antigen-pulsed presenting cells were prepared by incubating 1–2 μM mb and 10×10^6 tumor or spleen cells for 2 h. The cells were washed three times in Hanks' balanced salt solution, treated with either mitomycin-C or γ-irradiation, and assayed with the antigen-reactive T cells, as described above. The *d* haplotype cells used in these experiments are high responders to both mb (17) and GAT (18).

Antiserum Treatment of Antigen Proliferation Cultures. Alloantiserum inhibition of T cell proliferation cultures was done by mixing comparable amounts of specific anti-Ia or control sera in the T cell proliferation assay. Mb-specific T cell proliferation was shown to be independent of any residual Ia-positive cells in the T cell cultures by treating the T cell population with A.TH anti-A.TL serum and complement. The cells (10×10^6 to 20×10^6/ml) in medium 199 2% horse serum were mixed with 100 μl A.TH anti-A.TL for 30 min at 4°C, washed three times in medium 199 2% horse serum, and resuspended at 10×10^6 to 20×10^6 cell/ml in medium 199 containing 1:10 dilution of rabbit serum (complement source) for 1 h at 37°C. The treated cells were washed, counted, and added to the T cell proliferation assay. A similar anti-Ia plus complement treatment of DBA/2 spleen, L10A2J, or WEHI 5 cells was done in parallel to demonstrate the effectiveness of the anti-Ia/complement-mediated cytotoxicity.

Results

Soluble Antigen-reactive T Cells. The highly enriched antigen-reactive T cells used in these experiments were grown as long-term continuous cultures. Previous reports (16) on similarly derived antigen-reactive T cells have demonstrated that they are capable of providing T helper cell functions in an in vitro plaque assay (R. Hodes, and M. Kimoto, personal communication) and their proliferative response is antigen specific and MHC (I region) restricted. Table II shows that the DBA/2 mb-reactive T cells are only stimulated in the presence of antigen-presenting spleen cells that share the $H\text{-}2^d$ haplotype (DBA/2, B10.D2). The fact that neither B6 ($H\text{-}2^b$) nor A/J spleen cells ($K^k I\text{-}A^k\ I\text{-}B^k\ I\text{-}J^k\ I\text{-}E^k\ I\text{-}C^d\ S^d\ D^d$) present mb to the DBA/2 mb-reactive T cells provisionally maps the genetic restriction requirements of these T cells between K^d and $I\text{-}E^d$. Similarly derived mb-reactive B6A long-term cultured T cells and T cell

TABLE II

*MHC Restriction of DBA/2 α mb T Cells**

Antigen-presenting spleen cells of strain	[^{3}H]Thymidine incorporation	
	Without mb	With mb
DBA/2	1,640	28,673
B10.D2	1,965	28,372
B6	1,338	2,249
B6A	5,598	2,454
A/J	1,149	997

* Tumor or spleen cells were mixed (in triplicate) with 1 μM mb and 10^4 antigen-reactive T cells. [^{3}H]thymidine was added after 2 d of culture, and the cells were harvested on day 3.

TABLE III

Tumor Cell Antigen Presentation

APC	[^{3}H]Thymidine incorporation			
	DBA/2 α mb T cells		DBA/2 α GAT T cells	
	Without mb	With mb	Without GAT	With GAT
WEHI 265.1*	3,089	3,787	2,881	2,513
WEHI 3*	655	607	ND‖	
HUCL-1*	574	473	ND	
WEHI 5‡§	2,804	17,575	3,702	20,346
L10A2J‡	4,283	15,513	5,544	22,103
WEHI 231‡§	224	7,378	561	21,368
T2‡§	2,362	15,510	3,541	25,714
D2N‡§	1,666	13,715	5,719	24,956
WEHI 265‡	11,269	15,401	7,214	12,373
DBA/2 Spleen*	1,545	39,047	976	60,207

* Irradiated (3,300 rad).
‡ Mitomycin-C treated.
§ Tumor cells treated with mitomyin-C induced a similar amount of antigen-specific [^{3}H]thymidine incorporation with DBA/2 antigen-reactive T cells.
‖ Not done.

clones required sharing of the I-A or I-E and I-A subregion between the APC and T cells to generate antigen-specific T cell proliferation (A. Infante, unpublished results).

Selection of Tumor Cells That Present Antigen. In this laboratory, during the past several years, a number of tumor cells have been screened for the expression of Ia antigens by biochemically isolating intrinsically radiolabeled Ia molecules. These tumor cell lines were also assayed for their ability to present soluble antigen to antigen-reactive T cells in a T cell proliferation assay (Table III). Initial experiments indicated that the tumor cell lines pretreated with γ-irradiation or mitomycin-C allowed comparable levels of antigen-specific T cell proliferation (data not shown). The L10A2J line was an exception to this in that we were unable to inhibit intrinsic [^{3}H]thymidine incorporation with previous γ-irradiation. Consequently, the L10A2J line was always assayed after mitomycin-C treatment. Tumor cells that would not allow antigen-specific T cell proliferation could not be shown by biochemical analysis to express detectable levels of Ia antigens. One possible exception to this is WEHI

265, which expresses small amounts of Ia antigens and which induced only marginally significant antigen-specific T cell proliferation. The two tumor lines that were initially shown to allow antigen-specific T cell proliferation (L10A2J and WEHI 5) were used in all subsequent experiments to characterize the specificity of this antigen-presenting function.

Kinetics of Responses. To optimize the T cell proliferation assay with the tumor cells, either the amounts of antigen or the numbers of antigen-presenting tumor cells were titrated with a constant number of mb-reactive DBA/2 T cells. Fig. 1 A shows that when L10A2J, WEHI 5, or DBA/2 spleen cells are used to present varying amounts of mb to mb-reactive T cells, optimal T cell proliferation is produced with 1 μM mb. When the amount of mb and number of T cells were held constant and the number of tumor or spleen cells were varied, maximum T cell proliferation was produced with 10^5–10^6 spleen and 10^5 tumor cells (Fig. 1B). In both titration experiments, the tumor lines and spleen cells gave qualitatively and quantitatively similar dose-response curves.

Antigen-pulsed Presenting Cells. Previous studies (1) that used adherent macrophage-like cells as APC have shown that antigen-specific T cell proliferation could be generated if the antigen was either present throughout the assay period or if the APC were first incubated (pulsed) with the antigen, washed to remove unbound antigen, and then added to the antigen-reactive T cells. To determine whether these tumor cells required the constant presence of soluble antigen to generate antigen-specific T cell proliferation, we incubated the tumor cells with mb for 2 h and washed unbound antigen from the cells before adding them to the mb-reactive T cells. Table IV shows that the pulsed WEHI 5 tumor cells induced a T cell proliferative response that was equal to or better than the response generated when the antigen was present in the culture medium throughout the assay period. Similar results have also been obtained with pulsed L10A2J tumor cells (data not shown).

MHC Restriction. It has previously been demonstrated (6–8) in several different species that soluble protein antigen can be effectively presented to antigen-reactive T cells by an APC only if the T cell and APC share a common I-region allele. The

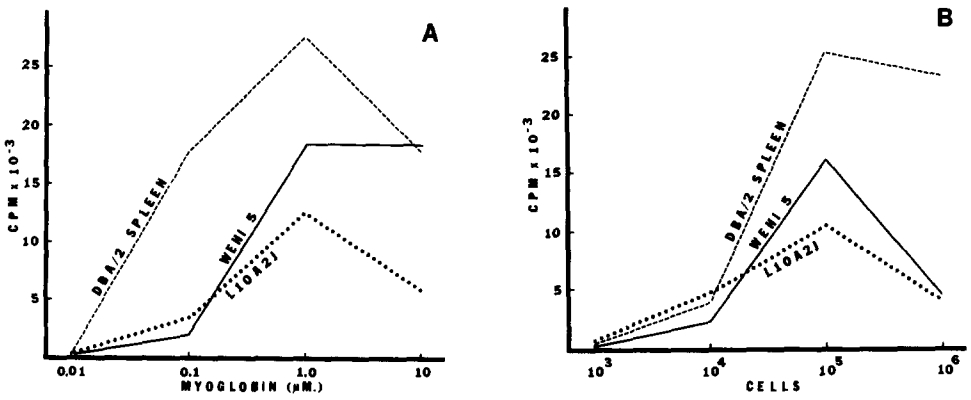

FIG. 1. Titration of the quantity of (A) mb and (B) antigen-presenting cells needed to generate maximum levels of antigen-specific T cell proliferation. In both experiments the antigen-presenting cells were treated with mitomycin-C before assay with 10^4 DBA/2 mb-reactive T cells.

TABLE IV

Anti-Ia Treatment of Different Cell Populations That Are Necessary for T Cell Proliferation Assay

APC	Antigen-reactive T cell (10^4)	^{3}H incorporated		
		Without mb	With mb	Antigen pulsed*
WEHI 5 (10^5)	DBA/2 α mb	2,535	12,519	20,705
WEHI 5 (10^5)	*DBA/2 α mb*‡	1,666	21,257	28,791
L10A2J (10^5)	DBA/2 α mb	5,695	10,467	ND§
L10A2J (10^5)	*DBA/2 α mb*‡	4,056	11,817	ND
DBA/2 spleen (10^6)	DBA/2 α mb	969	45,437	36,701
DBA/2 spleen (10^6)‡	DBA/2 α mb	435	4,384	ND
DBA/2 spleen (10^6)	*DBA/2 α mb*‡	1,651	64,637	ND
DBA/2 spleen (10^6)‡	*DBA/2 α mb*‡	319	9,594	ND

* Antigen-pulsed cells were incubated with 1 μM mb for 2 h, washed, irradiated, and added to the assay wells.

‡ Cells were treated with anti-Ia (A.TH α A.TL) (I-A^d + I-E^d) and rabbit complement before assay. The treatment killed 96+% of WEHI 5 and L10A2J tumor cells. Similar results have been obtained using (B10 × D2.GD) α B10.5R instead of A.TH α A.TL anti-Ia. Irradiated spleen, irradiated WEHI 5, or mitomycin-C treated L10A2J cells were mixed in triplicate with 1 μM mb and 10^4 DBA/2 mb-reactive T cells.

§ ND, Note done.

TABLE V

Antigen Presentation by Tumor Cells to Syngeneic and Allogeneic mb-reactive T Cells

	APC	Antigen-reactive T cell (10^4)	^{3}H incorporation	
			Without mb	With mb
A.	WEHI 5 (10^5)‡	DBA/2 α mb	1,283	29,013
	DBA/2 spleen (10^6)§	DBA/2 α mb	1,282	18,530
	WEHI 5 (10^5)‡	B6A α mb	1,581	930
	B6A spleen (10^6)§	B6A α mb	657	62,578
B.	L10A2J (10^5)‡	DBA/2 α mb	1,343	7,974
	LPS L10A2J (10^5)‡	DBA/2 α mb	2,213	12,115
	DBA/2 spleen (10^6)§	DBA/2 α mb	1,776	16,735
	L10A2J (10^5)‡	B6A α mb	1,211	1,449
	LPS L10A2J (10^5)‡	B6A α mb	1,774	1,685
	B6A spleen (10^6)§	B6A α mb	1,233	53,281

* Groups A and B were assayed on different days.

‡ Mitomycin C-treated (50 μg for 30 min).

§ Irradiated (3,300 rad).

antigen-reactive T cells are thought to recognize an I-region-encoded restriction site in addition to a specific antigenic determinant to generate T cell proliferation (6, 8, 16). To determine whether these tumor cells could present antigen in a similar MHC-restricted fashion, several different antigen-reactive T cell cultures, each with reactivity to different antigens and/or different I-region-encoded restriction sites, were stimulated with antigen and antigen-presenting tumor cells. The data in Tables III and V demonstrate that both L10A2J and WEHI 5 tumor cells can effectively present the appropriate antigen to DBA/2 mb-reactive T cells and DBA/2 GAT-reactive T cells but not to B6A mb-reactive T cells.

Studies done both in vitro (19, 20) and in vivo (21) have demonstrated that the addition of anti-Ia antisera to APC-T cell cultures can be used to block antigen recognition by T lymphocytes. If the antigen presentation function of WEHI 5 is similarly MHC-restricted, antigen-specific T cell proliferation should be blocked by the addition of anti-I^d antibodies to the proliferation assay. Because the responding T cells in our assay system are not cloned there could be T cells present that are restricted to Ia antigens encoded in the I-A subregion (A_β^d A_α^d) and other T cells restricted to Ia antigens encoded in I-E (A_e^d E_α^d) (Table II; and A. Infante, personal communication). Thus, monoclonal antibodies with reactivity to $I\text{-}A^d$ or $I\text{-}E^d$ subregion Ia antigens could be expected to only partially block mb-specific T cell proliferation. Alloantisera specific to all I^d-region Ia antigens should completely block the antigen-specific T cell proliferation. This predicted pattern of blocking of T cell proliferation by anti-Ia antibodies was observed when the WEHI 5 cells were used as APC (Table VI). The addition of either monoclonal anti-Ia.7 (I-E) or alloanti-Ia.23 (I-E) to the antigen presentation assay partially blocked T cell proliferation, whereas the addition of (B10.LG × B6)F_1 anti-B10.HTG (anti-I^d) serum completely blocked T cell proliferation. In contrast, comparable amounts of monoclonal anti-Ia.15 monoclonal (A_β^d A_α^d), anti-Ia.19 (unreactive with A_e^d E_α^d or A_β^d A_α^d), anti-$H\text{-}2D^d$ or normal mouse serum did not significantly block the antigen-specific T cell proliferation.

To clarify the site of action of the anti-Ia sera, the APC or the antigen-reactive T cells were treated with A.TH anti-A.TL and complement before they were added to the proliferation assay. This anti-Ia treatment killed >96% of the WEHI-5 or L10A2J tumor cells. When the DBA/2 spleen cells were treated with anti-Ia and complement, the antigen-specific T cell proliferative response was reduced by 91% (Table IV). In contrast, when the DBA/2 spleen, L10A2J, or WEHI 5 cells were used to present mb

TABLE VI

*Blocking WEHI 5 Antigen Presentation to mb-reactive DBA/2 T Cells with Antisera**

Antisera	^{3}H incorporation	Percent of control response‡
NMS§	18,562	100
Anti-D^d‖	20,098	108
Anti-Ia.19¶	16,546	89
Anti-Ia.15**	16,579	89
Anti-Ia.7‡‡	10,637	57
Anti-Ia.23§§	10,870	58
Anti-I^d.‖‖	328	1

* Antisera was added to the T cell proliferation assay at the beginning of the culture period.

‡ The percentage is equal to [^{3}H]thymidine incorporation of experimental/[^{3}H]thymidine incorporation of normal mouse serum control.

§ Normal DBA/2 mouse serum, 2 μl.

‖ A.TFR-1 anti-A.TL, 2 μl.

¶ 116.32 monoclonal, 5 μl (1 mg/ml).

** 17/227 monoclonal, 5 μl (1 mg/ml).

‡‡ 13/4 monoclonal, 5 μl (1 mg/ml).

§§ (B10.LG × C3H/HEJ)F_1 anti-C3H.OL, 2 μl.

‖‖ (B10.LG × B6)F_1 anti-B10.HTG, 2 μl.

to anti-Ia-treated mb-reactive T cells, there was no reduction in antigen-specific T cell proliferation. The apparent enhanced proliferative response (Table IV) obtained when the DBA/2 spleen or WEHI 5 APC were assayed with anti-Ia-treated T cells rather than untreated T cells has not been consistently observed.

Discussion

It is widely accepted that the cells that present soluble antigen to T helper cells in vitro and in vivo in an MHC-restricted fashion are radioresistant, adherent, phagocytic immunoglobulin (Ig)-negative, Fc γ-receptor-positive, and Ia-positive cells that belong to the macrophage lineage (22). Steinman and Nuzzenzweig (23) have presented evidence that suggests that lymphoid dendritic cells that are adherent, nonphagocytic, Ig negative, Fc-γ receptor-negative, and Ia-positive are also effective at presenting antigen. Data are presented in this paper that demonstrate that a series of Ia-positive tumor cells with the phenotypic characteristics of B lymphocytes can also present antigen in vitro to antigen-reactive T cells. The experiments presented in this paper were designed to examine whether antigen presentation by these tumor cells was functionally similar to antigen presentation previously attributed to macrophages. The data from these experiments suggest that the tumor cells can effectively bind soluble antigen and present it to antigen-reactive T cells in an MHC-restricted fashion.

One requisite function of an APC is the ability to physically bind soluble antigen independent of cytophilic antibody before antigen processing and subsequent presentation to antigen-reactive T cells (24–26). Adherent macrophage-like APC that have been incubated (pulsed) with the antigen for relatively short periods of time and washed free of unbound antigen will effectively generate antigen-specific T cell proliferation in vitro (1). The WEHI 5 (Table IV) and L10A2J tumor cells (data not shown) that have been pulsed with mb generated specific T cell proliferation that was 44–98% greater than the proliferative response produced when the antigen was present in solution throughout the assay period. In contrast, DBA/2 spleen cells pulsed with antigen under similar conditions usually produced a T cell proliferative response that was equal to or slightly less than that produced when antigen was present throughout the assay. The significance of this difference is not known. The ability to use pulsed tumor cells to generate antigen-specific T cell proliferation should, in future analyses, enable us to separate the biochemical events of antigen uptake and processing from antigen presentation and T cell recognition in this tumor cell antigen-presenting system.

The tumor cells tested for antigen presentation function are all of the $H\text{-}2^d$ haplotype (BALB/c, DBA/2, or (BALB/c × NZB)F_1 and therefore should potentially be capable of presenting antigen only to antigen-reactive T cells that share $H\text{-}2^d$. The data in Tables III and V demonstrate that although WEHI 5 and L10A2J can allow antigen-specific proliferation from DBA/2 mb-reactive T cells or DBA/2 GAT-reactive T cells, the same tumor cells cannot allow mb-specific proliferation of B6A mb-reactive T cells. If the antigen-reactive T cells were recognizing an I-region-encoded determinant on the tumor cells in addition to antigen, we should be able to block antigen-specific T cell proliferation with anti-Ia sera. The data in Table VI show that (B10.GD × B6)F_1 anti-B10.HGT (anti-I^d) completely blocks antigen-specific T cell proliferation when WEHI 5 cells are used as APC, whereas comparable

amounts of control sera (normal mouse serum or anti-$H\text{-}2D^d$) do not significantly decrease the T cell proliferative response when WEHI 5 cells are used as APC. Table VI shows that monoclonal anti-Ia.7 (I-E) or alloanti-Ia.23 (I-E) partially block the antigen-specific T cell proliferative response when the WEHI 5 cells are used as APC. The absence of significant blocking by the anti-Ia.15 monoclonal antibody could be due to the absence of a significant number of DBA/2 T cells reactive with the region of the I-A subregion Ia molecule that is physically adjacent to the Ia.15 alloantigenic determinant. Preliminary MHC restriction experiments on T cell clones isolated from our DBA/2 mb-reactive T cells suggests that there are at least some mb-reactive T cells restricted to $I\text{-}A^d$ as well as $I\text{-}A^d/I\text{-}E^d$ in our T cell population. Additionally, the WEHI 5 tumor cells are capable of presenting antigen to both types of MHC-restricted T cell clones (data not shown).

Schwartz et al. (27) published data that indicated that an Ia-positive BALB/c tumor cell (A20) could function in a peritoneal exudate T lymphocyte subpopulation (PETLES) assay to induce antigen-specific T cell proliferation. However, this T cell proliferation was eliminated if the antigen-reactive T cells were treated with anti-Ia and complement. Schwartz et al. (27) interpreted these results as indicating that the tumor cell was not presenting antigen directly to the antigen-reactive T cell but was transferring the antigen to an Ia-positive cell in the PETLES antigen-reactive T cell population. This second Ia-positive cell could presumably function to effectively present the antigen to the antigen-reactive T cells.

To determine whether a similar phenomena was occurring with the L10A2J or WEHI 5 tumor cells, we treated the antigen-reactive T cells with anti-Ia and complement before testing with spleen or tumor cells in the antigen-presentation assay. Data in Table IV show that such anti-Ia pretreatment of the antigen-reactive T cells showed no significant decreased proliferation when antigen was presented by the L10A2J, WEHI 5, or DBA/2 spleen cells. The effectiveness of the anti-Ia treatment was demonstrated by similarly treating either the DBA/2 spleen cells, L10A2J, or WEHI 5 tumor cells with anti-Ia and complement. Greater than 96% of either the L10A2J or WEHI 5 tumor cells were killed by this treatment. Anti-Ia treatment of the DBA/2 antigen-presenting spleen cells killed 40–50% of the DBA/2 spleen cells and decreased the antigen-specific T cell proliferative response by >90%. These data indicate that if there are any residual Ia-positive cells in our antigen-reactive continuous T cell cultures, they do not play a significant role in antigen presentation by the tumor cells. These data also demonstrate that the target cell in the anti-Ia blocking of T cell proliferation (Table IV) is probably the APC and not the responding T cell.

An important question that remains unanswered concerns the biological significance of antigen presentation by normal Ia-positive lymphocytes. These five tumor lines, which can be shown to synthesize Ia antigens, all allow antigen-specific proliferation in antigen-reactive T cells. The phenotypic markers expressed by these cells (Table I, reference 28) indicate that four of the five lines are not of the macrophage lineage. Although there have been previous reports (1, 29, 30) suggesting that highly enriched populations of B lymphocytes were capable of presenting antigen to T cells, the presence of small numbers of contaminating macrophage-like APC could never be excluded. The data presented in this paper suggest that a normal lymphocyte subpopulation represented by these tumor cell lines may exist in vivo that are capable of presenting antigen to T helper cells.

The titration of the numbers of spleen cells or tumor cells required to generate a maximum T cell proliferative response (Fig. 1) suggests that an equal number of spleen or tumor cells are needed to generate a comparable T cell proliferative response. Because macrophages represent only a small percentage of spleen cells, these titration data appear to suggest that the tumor cells are not as efficient on a per cell basis at presenting antigen. Although this conclusion might prove to be correct, the potential heterogeneity of the tumor cells should be considered before it is concluded that the antigen presentation phenomena exhibited by these tumor cells has no biological significance. First, these tumor lines that have been grown in in vitro culture for several years are not cloned cells. Second, we have observed that at least some of the tumor lines have the tendency to modulate their levels of expression of Ia antigens when maintained in in vitro culture for extended periods. For example, the WEHI 265.1 subline was maintained in in vitro culture for several years and lost its ability to express Ia antigens. The parent WEHI 265 line that was recovered after being frozen clearly expresses Ia. The tumor cell lines are currently being subcloned and will be tested to determine whether the level of Ia antigen expression or other phenotypic markers can be correlated with the cell's ability to effectively present antigen.

The tumor cell lines might, on the other hand, be deficient in certain functions (e.g., mediator production) that are expressed by macrophages or possibly third party cells and are necessary for optimal antigen presentation to antigen-reactive T helper cells. For example, when WEHI 5 or L10A2J tumor cells were stimulated with LPS for 2 d before being used as APC, the antigen-specific T cell proliferative response to DBA/2 mb-reactive T cells was increased by 40–50% (Table V). This was shown not to be a nonspecific effect of residual LPS inasmuch as B6A mb-reactive T cells were not stimulated by the LPS-treated L10A2J cells. LPS stimulation increased the total amount of Ia antigens synthesized by L10A2J and WEHI 5 cells by an average of 50% (D. J. McKean, unpublished observations) and also might potentially increase the expression of mediators (31). Experiments are in progress to determine whether cloned tumor APC require other soluble mediators to more effectively present antigen to cloned antigen-reactive T cells.

Tumor cells have historically provided a means of viewing functions of select subpopulations of cells within the immune system. The results of the experiments in this report demonstrate that WEHI 5 and L10A2J tumor cells exhibit the major functions previously attributed to macrophage APC. They are capable of binding antigen and presenting the cell-associated antigen in conjunction with Ia determinants to antigen-reactive T cells. Once these tumor cells are cloned, they should provide a valuable model system for examining the biochemical basis for antigen uptake, processing, and Ia-associated antigen presentation to cloned antigen-reactive T cells. The data presented in this paper also suggest the need for a critical examination of normal subpopulations within the murine immune system to determine exactly which cells can present soluble antigen.

Summary

Previous reports have demonstrated that accessory cells function to present soluble protein antigens in association with gene products encoded within the I region of the major histocompatibility complex (MHC) to antigen-reactive T helper cells. The

biochemical events that occur during antigen presentation are, however, not well-documented primarily because of the difficulties involved in purifying sufficient numbers of homogeneous antigen-presenting cells. In this paper, a number of Ia-positive B lymphocyte tumor lines are shown to be capable of presenting soluble protein antigens to antigen-reactive continuous T cell lines in an MHC-restricted fashion. The characterization of the antigen presentation function of these tumor cells indicates that the tumor cells have many of the functional antigen-presenting characteristics previously thought to be limited to macrophages. These tumor cells should provide a useful model system for determining the biochemical events that occur in antigen uptake and processing as well as for determining the potential interactions between processed antigen and Ia molecules on the plasma membrane of these antigen-presenting cells.

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