Brief Definitive Report

SPECIFIC ADSORPTION OF IgM ANTIBODY ONTO H-2-ACTIVATED MOUSE T LYMPHOCYTES

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Transfer of T cells into irradiated H-2-incompatible mice is followed 4 days later by the appearance of large numbers of donor-derived, θ-positive, blast T cells in thoracic duct lymph (T.TDL) (1). Virtually all T.TDL carry surface immunoglobulin (Ig) (2–4). When T.TDL were generated from T-cell populations depleted of B cells, the surface Ig on T.TDL was considerably reduced (4). It was concluded therefore that at least a proportion of the Ig detected on T.TDL was alloantibody of donor B-cell origin and might be attached via soluble host alloantigens bound by antigen-specific receptors.

Two predictions follow from this hypothesis: first, that T.TDL would be Ig negative when raised against determinants which stimulate T cells but not B cells, for example, *M*-locus determinants (5); and second, that T.TDL activated to H-2 determinants (though not to *M*-locus determinants) would adsorb specific alloantibody when incubated with appropriate alloantisera in vitro. This paper presents data which are in accord with these predictions.

Materials and Methods

Cells. T.TDL were generated against H-2 and *M*-locus differences by the transfer of thymocytes between strain combinations as shown in Table I: activated cells were collected by cannulation of the thoracic duct (1) at 4 or 5 days later. In some experiments thymocytes were depleted of Ig-bearing (B) cells by anti-Ig column filtration (6).

Antisera

ANTI-Ig. SERA. Antisera were raised in rabbits against mouse Ig subclasses (7) and rendered specific by cross-absorption (8). Purified anti-Ig antibodies were raised as described elsewhere (9).

ALLOANTISERA. To raise hyperimmune CBA anti-C57BL alloantiserum, CBA/J mice were given five injections of 3×10^7 C57BL spleen cells at fortnightly intervals and bled 2 wk after the last injection. This serum killed >95% of (CBA/J × C57BL)F_1 lymph node cells in the presence of guinea pig complement.

"Early" CBA anti-C57BL alloantiserum was prepared by injecting CBA/J mice with 2×10^8 (CBA/J × C57BL)F_1 spleen cells and bleeding the recipients 5, 6, or 7 days later. To remove activity against public H-2 specificities present on DBA/2 cells, the sera were absorbed extensively with DBA/2 spleen cells before use. Similarly, early CBA anti-DBA/2 serum was raised in CBA/J mice injected with (CBA/J × DBA/2)F_1 spleen cells and the antiserum absorbed with C57BL spleen cells.

Incubation of T.TDL in sera. Aliquots of 10^7 T.TDL were incubated (30 min at 37°C) in a 1:4 dilution of the relevant antiserum containing 20 mM sodium azide and washed three times by centrifugation before immunofluorescent staining (4).

* Holder of a C. J. Martin Travelling Fellowship.

TABLE I

Percentage of Immunoglobulin-Positive Cells in T.TDL Activated either to H-2 Determinants or M Locus Determinants: Cells Collected from Lymph at 4–5 days after Thymus Cell Transfer

Transferred* cells	X-irradiated recipients	Activating determinants	% Fluorescent T.TDL with‡		
			Anti-Ig antibodies	Anti-IgM	Anti-IgG_1
Normal CBA/J thymus	(CBA/J × C57BL)F_1	H-2^b + Mls^b	59 ± 21 (14)	34 ± 6 (6)	4 ± 2 (6)
B-depleted CBA/J thymus	(CBA/J × C57BL)F_1	H-2^b + Mls^b	7 ± 8 (10)	2 ± 2 (6)	6 ± 4 (3)
B-depleted CBA/J thymus + TxBM CBA/J lymph node§	(CBA/J × C57BL)F_1	H-2^b + Mls^b	69	24	4
Normal CBA/J thymus	(CBA/J × DBA/2)F_1	H-2^d + Mls^a	75	ND‖	ND
B-depleted CBA/J thymus	(CBA/J × DBA/2)F_1	H-2^d + Mls^a	7	ND	ND
Normal C3H/J thymus	CBA/J	Mls^d	7 ± 7 (6)	0.9	3
Normal BALB/c thymus	DBA/2	Mls^a	5 ± 6 (3)	2.5	6

* 2×10^6 cells transferred intravenously into X-irradiated (800 R) mice.

§ 2×10^6 cells from thymectomized, irradiated bone marrow reconstituted mice. Cytotoxic index with AKR/J anti-θC3H serum and complement (10) was <3%; this antiserum lysed >75% of lymph node cells prepared from normal mice.

‡ Data as mean ± SD. Number of observations in parenthesis, or mean of two experiments.

‖ ND, not determined.

Results

Surface Ig, as detected by indirect immunofluorescence, was present on approximately 60% of T.TDL obtained from irradiated (CBA/J × C57BL)F_1 mice injected 4–5 days previously with CBA/J thymus cells (Table I). A similar proportion of DBA/2-activated CBA/J T.TDL were Ig positive. When stained cells were incubated at 37°C before counting, over 90% of the blast cells showed immunofluorescent caps, suggesting that all the blast cells probably carried small amounts of surface Ig. Removal of B cells from the thymocyte progenitors caused up to a 10-fold reduction in the number of Ig-positive T.TDL (Table I). This reduction was not observed when the B-cell-depleted thymus cells were injected together with 2×10^6 B cells (lymph node cells from TxBM CBA/J mice).

Table I also shows that T.TDL raised between strains differing only at the *M* locus contained very few Ig-positive cells; this applied both with CBA/J (Mls^d)-activated C3H/J T.TDL and DBA/2 (Mls^a)-activated BALB/c T.TDL.

When the activated cells were collected at 5–6 days, both H-2-activated and *M*-locus-activated T.TDL contained a high proportion (40–50%) of Ig-positive cells (Table II). Staining with subclass-specific antisera showed that the Ig on the *M*-locus-activated cells was almost entirely IgG (IgG_1 and IgG_{2a}, though not IgG2b). On H-2-activated T.TDL, however, the surface Ig consisted of a mixture of IgM and IgG.

To investigate whether H-2-activated T.TDL could bind alloantibody in vitro, C57BL-activated CBA/J T.TDL derived from B-cell-depleted thymus cells were incubated in vitro with either normal mouse (CBA/J) serum, hyperimmune CBA/J anti-C57BL antiserum, or "early bleed" CBA/J anti-C57BL antiserum;

TABLE II

Class Distribution of Immunoglobulin on T.TDL Activated either to H-2 Determinants or M-Locus Determinants: Cells Collected from Lymph at 5–6 days after Thymus Cell Transfer

Transferred cells*	X-irradiated recipients	% Positive T-TDL after incubation in antiserum to:						
		IgM	IgA	IgG_1	IgG_{2a}	IgG_{2b}	NRIgG‡	Ig (purified antibody [9])
CBA/J thymus	(CBA/J × C57BL)F_1	40.0 ± 27	2.8 ± 2.9	48 ± 5.7	21 ± 11	8.5 ± 2.5	6.5 ± 2.4	56 ± 8.2§
CBA/J thymus, B-cell depleted	(CBA/J × C57BL)F_1	5.0	ND‡	26	ND	ND	4.1	29
C3H/J thymus	CBA/J	1.8 ± 0.8	0.3 ± 0.3	38 ± 3.7	46 ± 20	2.9 ± 0.1	4.1 ± 0.2	41 ± 3.0

* 2×10^8 cells transferred intravenously.
‡ NRIgG, normal rabbit IgG; ND, not determined.
§ Data as mean ± SD. Where SD is shown, mean of four observations, otherwise mean of two observations.

TABLE III

Capacity of (a) H-2-Activated T.TDL Derived from B-Cell-Depleted Thymus and (b) M-Locus-Activated T.TDL to Adsorb IgM and IgG_1 from Alloantisera and Normal Serum In Vitro

T.TDL incubated at 37°C for 30 min in:	T.TDL			
	CBA/J anti-C57BL		C3H/J anti-CBA/J	
	% Stained with*: Anti-IgM	Anti-IgG_1	% Stained with*: Anti-IgM	Anti-IgG_1
1:4 dilution, 5 day CBA anti-C57BL serum‡	32	23	4	37
1:4 dilution, 6 day CBA anti-C57BL serum	36	29	9	20
1:4 dilution, 7 day CBA anti-C57BL serum	4	34	4	29
1:4 dilution, hyperimmune CBA anti-C57BL serum‡	4	21	7	13
1:4 dilution, normal CBA serum	7	26	1	34
1:4 dilution, 7 day CBA anti-DBA/2 serum‡	2	21	0	46
Medium alone, at 4°C	2	5	0	3

* By indirect immunofluorescence T.TDL collected at 4–5 days after thymus injection.
‡ Antisera raised as described in Materials and Methods.

the latter was obtained at 5, 6, or 7 days after initial sensitization, i.e., at a stage when most of the antibody would presumably be IgM (see Materials and Methods).

T.TDL bound IgG (IgG_1) irrespective of the serum used for incubation (Table III). Uptake of IgM, however, was only observed with the early bleed antirecipient antiserum and then only when the latter was obtained at 5 or 6 days postsensitization. Uptake of IgM from these antisera was either very low (Table III) or absent (Table IV) with *M*-locus-activated (CBA/J-activated) C3H/J T.TDL.

The specificity of the uptake of IgM by T.TDL is shown in Table IV. C57BL-activated CBA/J T.TDL bound IgM when incubated with 6-day bleed CBA/J anti-C57BL antiserum but not with CBA/J anti-DBA/2 serum. Reciprocal results were obtained with DBA/2-activated CBA/J T.TDL. *M*-locus-activated T.TDL did not adsorb IgM from either antiserum. Again, IgG was adsorbed nonspecifically by all three cell populations.

TABLE IV
Specificity of In Vitro Uptake of IgM and IgG_1 onto T.TDL

T.TDL*	Activating determinants	T.TDL incubated at 37°C for 30 min in 1:4 dilution of:	% Stained with:	
			Anti-IgM	Anti-IgG_1
CBA/J anti-DBA/2	H-2^d + Mls^a	CBA anti-C57BL serum	2	77
		CBA anti-DBA/2 serum	33	41
		Medium alone	3	3
CBA/J anti-C57BL	H-2^b + Mls^b	CBA anti-C57BL serum	13	40
		CBA anti-DBA/2 serum	1	43
		Medium alone	2	3
C3H/J anti-CBA/J	Mls^d	CBA anti-C57BL serum	3	30
		CBA anti-DBA/2 serum	3	49
		Medium alone	2	5

* T.TDL derived from B-cell-depleted thymus; cells collected day 4–5.

Discussion

The present paper presents three pieces of evidence which support the hypothesis that the IgM detected by indirect immunofluorescence on T.TDL represents specific alloantibody of donor B-cell origin. First, IgM was present on H-2-activated T.TDL derived from normal thymus (Table I) or lymph node cells (unpublished data), but not when these cells were depleted of B cells before injection (Table I); this deficit of IgM-positive T.TDL was not observed when the B-cell-depleted precursors were supplemented with small numbers of B cells. Second, surface IgM was not detected on T.TDL raised between strains differing only at the *M*-locus (Tables I and II). Third, H-2-activated T.TDL bound IgM specifically when incubated with antirecipient (but not antithird-party) alloantisera in vitro (Tables III and IV); this was only observed with sera obtained soon after initial sensitization, i.e., at a time when the alloantibodies were presumably IgM rather than IgG.

It was suggested previously that specific IgM antibody on T.TDL is attached to antigen-specific receptors on the cells via a bridge of soluble host alloantigen (4). How then can one explain the capacity of IgM-negative H-2-activated T.TDL (i.e., T.TDL derived from B-cell-depleted T cells) to adsorb IgM from specific alloantisera in vitro? There would appear to be two main possibilities. First, the IgM adsorbed from the alloantisera might have been in the form of immune complexes; in this situation the complexes could become attached (via the antigen) to alloantigen-specific receptors on the cells. Second, the IgM could have been free antibody which attached to cells carrying receptor-bound alloantigens not complexed with antibody. We cannot at present distinguish between these two possibilities. Nevertheless, it is intriguing to consider that if the second possibility were correct, i.e. the specific receptors on the cells were blocked, it might explain why T.TDL enter the lymph of the host rather than remaining sequestered in the alloantigen-rich environment of the lymphoid tissues.

Preliminary experiments have shown that large numbers of T.TDL can be generated between strains differing only at the *I* region of the H-2 complex (J. Sprent, L. Hudson, M. Nabholz, and H. von Boehmer, unpublished data). When these T.TDL were generated from normal lymph node cells, a large proportion

were Ig positive. This was not observed, however when the T.TDL were derived from B-cell-depleted lymph node cells. Hence much of the specific antibody detected on T.TDL could represent anti-*I*-region (anti-Ia) antibody complexed to host-type Ia antigens.

Much of the IgG detected on T.TDL appeared to bind nonspecifically. With both H-2-activated and *M*-locus activated populations, the cells carried surface IgG when collected from the lymph at day 5–6 but not at day 4–5. The cells collected at day 4–5, however, were able to bind IgG when incubated with normal mouse serum (and other sera) in vitro. There are several reports that at least certain T-cell populations express Fc receptors (FcR) for IgG (e.g. 11, 12). It could be argued therefore that T.TDL express FcR which are of very low affinity when collected at day 4–5 but of higher affinity at day 5–6. The adsorption of IgG from normal mouse serum in vitro by the day 4–5 cells was only observed with relatively high concentration (1:4 dilution) of serum (Table III, and unpublished data); such conditions might be optimal for detecting FcR of low affinity. It is nevertheless puzzling that T.TDL collected at this stage failed to adsorb IgG from the lymph, the concentration of IgG in lymph being, at least in rats (13), only three- to four-fold lower than in serum. Even more puzzling, however, is the failure of our own and other groups to demonstrate FcR on T.TDL by conventional (14, 15) techniques. Further investigation will be necessary to resolve this paradox.

Since in the case of the day 5–6 H-2-activated T.TDL the numbers of IgG-positive cells were partly reduced when the precursors were depleted of B cells (Table II), some of the IgG detected on the cells might have been specific antibody. Although it is unlikely that this accounted for the IgG on *M*-locus-activated T.TDL, this possibility has yet to be excluded.

It was suggested previously that FcR might stabilize the binding of specific IgM antibody to T.TDL (4). Although in the present study we failed to demonstrate nonspecific uptake of IgM by T.TDL, the cells might express FcR of low avidity for IgM. The recent report that thymus cells express FcR for IgM after culture in vitro (16) is at least consistent with this view.

Summary

Evidence is presented to support the contention that IgM demonstrable by surface immunofluorescent staining on H-2-activated T cells represents specifically adsorbed B-cell-derived alloantibody. T cells activated to H-2 determinants expressed surface IgM only when the progenitor cell populations contained B lymphocytes. IgM was not detected on T cells activated to determinants which fail to stimulate alloantibody production (e.g., *M*-locus determinants). In addition, IgM-negative H-2-activated T cells (derived from B-cell-depleted lymphoid cells), unlike *M*-locus-activated T cells, adsorbed IgM in a specific manner when incubated in vitro with "early bleed" antisera raised against the activating H-2 determinants.

Received for publication 29 September 1975.

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