IMMUNE RESPONSES IN VITRO

IX. ABSENCE OF THE MIXED LEUKOCYTE STIMULATORY, M-LOCUS PRODUCT FROM THYMUS CELLS

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The principal obstacle to successful clinical transplantation of a human organ, immunological rejection, seems to be a result of inadequacies in two areas: (*a*) the inability to induce tolerance to tissue antigens; and (*b*) the inability of present donor-recipient matching schemes to differentiate major histocompatibility antigens from other cell-associated antigens. The importance of surmounting the latter stems from the fact that the immune activity against nonmajor histocompatibility antigens is relatively easily controlled via immunosuppressive therapy, whereas, the immune activity incited by major histocompatibility antigens primarily those encoded by the FOUR end (1, 2) of the HL-A locus (*H-2K* region of the mouse [3]) is very difficult to control.

Presently, two methods are used in assessing the relationship of histocompatibility antigens of donors and recipients for organ transplantation; tissue typing (serology) and the mixed leukocyte reaction (MLR) assays. A third, the release of ^{51}Cr from target cells by killer cells produced in one-way MLR, termed "cell-mediated lympholysis or CML" (4), is presently under investigation (5).

Statistically, MLR testing and serotyping correlate to homograft reactivity (6). However, numerous anomalies of MLR reactivity and serotyping result in a state in which neither technique accurately predicts the success or failure of graft survival before transplantation. Thus, successful transplantation of kidney grafts has been frequently achieved between familial-unrelated serologically nonidentical individuals (7–9); and transplantation of bone marrow as a therapeutic protocol for leukemia between serologically as well as MLR identical siblings has in many cases been unsuccessful because of a fatal graft vs. host reaction (10–13). Of these two techniques, evidence is, however, accumulating which suggests that the MLR will be of more value (14, 15) in predicting the clinical outcome of organ transplants.

The major disadvantages of the MLR are that it is unclear as to the nature of the antigens that induce stimulation. In the mouse model nonmajor histocompatibility antigens can cause stimulation (16–18), in some cases equal in magnitude to major histocompatibility induced stimulation (16–18). There-

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fore, to increase the value of the MLR for tissue matching, it seems imperative to define the antigens that induce MLR activity and to be able to differentiate major histocompatibility antigens from other cell-associated antigens. It may also be of importance to differentiate these other nonmajor alloantigens from each other.

Because of the multitude of mouse strains which have been genetically defined, plus the apparent homology of the major histocompatibility locus of man (HL-A) and mouse (*H-2*) (19), this species should be the most valuable in establishing assays which measure antigens that are of major importance in transplantation. Utilizing a dual MLR system, one in a chemically defined protein-free medium (17) and the other in a mouse serum supplemented medium (20, 21), stimulation by alloantigens associated with the *H-2* region has been differentiated from stimulation by two non-*H-2* alloantigens (18). Moreover, stimulation due to these two non-*H-2* alloantigens, the theta antigen of thymus or thymus-derived cells (22) and the product of the M-locus (16), can be distinguished from each other (18, 22).

The results of the present report are an extension of our investigations on the definition of nonmajor histocompatibility antigens which induce MLR reactivity. Specifically, the results indicate that the stimulatory product of the M-locus is not present on thymus cells. The significance of this finding is discussed in relationship to the CML assay in which phytohemagglutinin-stimulated cells (T cells) are the ^{51}Cr-labeled target cells.

Materials and Methods

Mice.—Inbred strains used in this study and raised in this laboratory are BALB/cHeAu, C3H/HeAu, and DBA/2J. Both male and female mice were used, ranging in age from 5 to 7 wk. Within any one experiment, the sex and age of the animals were the same.

MLR Conditions.—Preparation of cells, X irradiation of the stimulating cell populations, culture conditions, and determination of [^{3}H]thymidine incorporation are detailed in other reports (17, 21). The protocol for preparation of mouse serum is also detailed elsewhere (20). Spleen cells, thymus cells, and sera were always pools of three or more mice. The culture medium (obtained from Altick Assoc., Madison, Wis.) was supplemented to contain 0.5% autologous (to the responding strain) serum.

The results presented are representative of those obtained in many experiments. Counts per minute are the means of triplicate cultures. Stimulation indices are the ratios of [^{3}H]TdR incorporated in allogeneic reactions to that incorporated in syngeneic reactions.

Sufficient inactivation of the stimulating cells by X irradiation was assessed in each experiment by culturing together X-irradiated cells from each of two allogeneic populations. [^{3}H]TdR incorporation by these X-irradiated controls was less than 10% of the syngeneic controls. This was considered adequate inactivation.

RESULTS

Table I presents results which confirm the reported reactivity of BALB/c spleen cells against DBA/2 (M-locus products) and C_3H (*H-2* alloantigens) spleen cells (16–18). BALB/c thymus cells were also found to be stimulated by spleen cells of the same two strains.

TABLE I

MLR Stimulatory Capacity of H-2 and Non-H-2 Alloantigens Present on Spleen and Thymus Cells

Reacting strain*	Stimulating strain‡	Sources of stimulating cells	CPM ± SE at:		Stimulation index at:	
			72 h	98 h	72 h	98 h
BALB/c spleen	BALB/c	Spleen	2,922 ± 92	2,801 ± 130	—	—
	DBA/2	Spleen	27,471 ± 625	24,323 ± 2,850	9.4	8.7
	C3H	Spleen	37,345 ± 930	56,849 ± 1,380	12.8	20.3
	BALB/c	Thymus	2,439 ± 79	2,741 ± 170	—	—
	DBA/2	Thymus	1,940 ± 56	2,823 ± 290	0.8	1.0
	C3H	Thymus	14,713 ± 990	22,690 ± 2,090	6.0	8.3
C3H spleen	C3H	Spleen	3,226 ± 4	3,949 ± 30	—	—
	DBA/2	Spleen	64,619 ± 1,000	61,510 ± 3,200	19.7	15.5
	C3H	Thymus	4,550 ± 350	5,193 ± 338	—	—
	DBA/2	Thymus	8,895 ± 380	12,908 ± 4	2.0	2.3
BALB/c thymus	BALB/c	Spleen	290 ± 40	230 ± 50	—	—
	DBA/2	Spleen	17,365 ± 1,020	15,857 ± 500	60.0	68.9
	C3H	Spleen	3,160 ± 200	1,719 ± 10	11.0	7.5

* 1.5×10^6 cells.
‡ 2.5×10^6 cells.

In contrast to the stimulatory properties of DBA/2 spleen cells, thymus cells of this strain did not stimulate BALB/c spleen cells. BALB/c cells were, however, stimulated by thymus cells of the *H-2* disparate strain, C_3H. Although not to the extent of spleen cells, DBA/2 thymus cells stimulated spleen cells of C_3H, an *H-2* disparate strain.

An apparent conflict between the present and our previous results (18), which is related to the relative stimulation observed at 72 and 98 h of culture due to *H-2* and M-locus differences, is because 2.5×10^6 stimulator cells were used in the present study while the optimal number of 2×10^6 were used in the previous experiments. This change was made so that the number of thymus and spleen cells used as antigen was constant.

In other immune systems, evidence is accumulating which indicates that T cells have a suppressor function (23–27). An experiment, therefore, was designed to test whether the absence of stimulation by DBA/2 thymus cells was due to a similar phenomenon. The results presented in Table II indicate that addition of 1×10^6 DBA/2 X-irradiated thymus cells neither stimulated nor inhibited the activity of BALB/c cells against 2.5×10^6 DBA/2 spleen cells. Addition of 2×10^6 DBA/2 thymus cells were inhibitory, affecting both the syngeneic as well as the allogeneic reaction. This inhibition was, however, a result of suppression by excessively high numbers of stimulating cells, as demonstrated previously with spleen cells (21).

TABLE II

BALB/c Spleen Cell Reactivity Against DBA/2 Spleen Cells in the Presence and Absence of Thymus Cells

X-irradiated spleen cells from:	No. of DBA/2 thymus cells	CPM at 72 h	Stimulation index
BALB/c	none	2,971	—
DBA/2	none	16,140	5.4
BALB/c	1×10^6	2,548	—
DBA/2	1×10^6	13,235	5.2
BALB/c	2×10^6	1,309	—
DBA/2	2×10^6	4,833	3.7

DISCUSSION

Transplantation has long offered the potential for correction of disease in which organ failure occurs. The major obstacle to successful organ transplantation is rejection. The success of immunosuppressive therapy employed to curtail rejection is related to the strength of antigen disparity (1–3). Since the techniques employed to assess disparity do not accurately predict success or failure of graft survival before transplantation, either new techniques and/or modifications of existing procedures must be sought. The present findings relate to one of the existing techniques, the MLR.

It is generally considered that the MLR is primarily mediated by thymus-derived (T) cells. However, recent evidence employing chromosome markers indicates that bone marrow (B) cells also proliferate in the presence of allogeneic cells (28). In experiments designed to identify the MLR stimulatory cells, conflicting findings have been reported. Lymphoid cells from neonatally thymectomized mice and rats have reduced stimulatory ability suggesting that T lymphocytes may be required as stimulator cells (29), or that the stimulatory cell is under thymic influence (30). In contrast, thoracic duct lymphocytes (TDL) of nu/nu mice (>97% B cells) were as stimulatory as TDL from nu/+ or +/+ littermates (80% T cells) (31); cells treated with anti-theta serum from spleens of thymectomized, X-irradiated adult mice reconstituted with B cells (T-cell depleted) stimulated as well as spleen cells from untreated animals (32); and T-cell depleted spleen cells (treated with anti-theta antiserum) were better stimulators than "B-cell" depleted populations obtained by treating with anti-Ly 4.2 antiserum (33). The results presented herein in which thymus cells were not as stimulatory as cells derived from the spleens of *H-2* disparate strain combinations support the observations that the *H-2* antigens of T cells are not as stimulatory as those of B cells. Although the rationale for these discrepancies is unclear, different culture conditions and strains of animals (all combinations were *H-2* disparate) were used by each investigator.

In the mouse, alloantigens other than those encoded by the *H-2* complex are stimulatory in the MLR. For stimulation incited by theta alloantigens (22), thymus or T cells, but not B cells, would be expected to cause stimulation. In

contrast, as shown in the present report, stimulation incited by disparity at the M-locus is not a result of thymus alloantigens. Thymus cells were, however, responsive to M-locus products. It is not known whether stimulation is a property of B cells, T cells, or some other cell type present in the spleen.

The findings of others (31–33) as well as those in this report in which thymus or T cells were found to either lack or possess diminished MLR stimulatory properties raise an important consideration for any hypothesis advanced to explain the divergent specificity of the induction (MLR dependent) and the effector (CML which is dependent on HL-A incompatibility) phases of cell-mediated immunity (34–36). That is, are the alloantigens controlled by the various MLR loci quantitatively and qualitatively expressed similarly on both the cell type(s) that stimulate in the MLR and the cell type (PHA-transformed T cells) employed as the target cell in CML? The unique expression of an M-locus allele on the cell type(s) that induce MLR stimulation and its absence from thymus cells (which can explain the lack of CML against this antigen [37]) make it imperative to answer this question before further hypotheses.

SUMMARY

The mixed leukocyte reaction stimulatory M-locus product of DBA/2 spleen cells was absent from thymus cells. The absence of stimulation was not a result of suppressor T cells. These findings are discussed in relation to the divergent specificity found for the inductive and effector phases of cell-mediated responses.

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