STIMULUS-RESPONSE IN THE MIXED LYMPHOCYTE REACTION

By MICHAEL R. HARRISON* and WILLIAM E. PAUL

(From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014)

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The proliferative response of allogeneic lymphocytes cultures together, the mixed lymphocyte reaction (MLR), has been widely used as an in vitro correlate of the antigen recognition phase of the allograft response. The MLR is most informative when it is unidirectional, so that any proliferative response measured in the mixed culture can be attributed to the capacity of one cell to respond (the responder cell) and another cell to stimulate a response (the target cell). The reaction is rendered "one-way" by inactivating the target cell with mitomycin C (1, 2) or irradiation (3, 4) such that it should not be able to contribute to the DNA synthesis used to measure the reaction. In inbred species, the mixture of parental and F_1 hybrid cells has been used as a natural one-way reaction, since the F_1 target cell should be genetically incapable of reacting to a cell of parental genotype (5–7).

In recent studies of the MLR, two puzzling sets of observations have been made: (*a*) mouse spleen cell populations depleted of thymus-dependent (T) lymphocytes or spleen cell populations obtained from athymic (*nu/nu*) mice appear to respond quite vigorously to mitomycin C-treated or X-irradiated allogeneic cells (8), and (*b*) F_1 hybrid spleen cells appear to respond to similarly inactivated parental target cells (9). The apparent responsiveness of T cell-deficient spleen populations is surprising because there is considerable evidence that the ability to respond is dependent on the presence of T lymphocytes (5–7, 10, 11). Similarly, the reported responsiveness of F_1 cells to parental cells runs counter to the conventional view that F_1 cells possess all the major alloantigens of each parent and are therefore genetically incapable of responding to parental strain cells (12, 13). The controversial nature of the above observations prompted a closer examination of the cellular interactions occurring in the one-way MLR.

Materials and Methods

AKR/J ($H\text{-}2^k$), DBA/2 ($H\text{-}2^d$), and (AKR × DBA/2)F_1 (F_1) mice were obtained from Jackson Laboratory, Bar Harbor, Me. 6–10-wk old AKR mice were thymectomized, irradiated (700 R), and reconstituted with 7×10^6 syngeneic bone marrow cells 3–6 wk before sacrifice, at which time the mediastinum was explored for residual thymic tissue. Additional in vivo ablation of T cells was accomplished by treatment of such mice with burro antimouse thymocyte antiserum, 0.5 ml intraperitoneally 3 and 2 days before sacrifice. MLR's were performed as previously described (14). 10^6 untreated responder spleen cells were cultured with 10^6 mitomycin-treated or irradiated stimulator cells in 0.2 ml of RPMI 1640 supple-

* Present address: Department of Surgery, Massachusetts General Hospital, Boston, Mass. 02114.

mented with antibiotics and 5% fetal calf serum in round-bottom microtiter wells. Cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere for 72 h. 16 h after addition of 1 μCi of [^{3}H]thymidine ([^{3}H]TdR) to each well, cells were harvested using a microculture harvesting device designed by one of us (M. R. H.), and the amount of radioactivity incorporated into acid-precipitable material was determined by scintillation spectrometry.

RESULTS AND DISCUSSION

Table I shows that spleen cells from T-deprived AKR mice (AKR^{-T}) are unresponsive to both phytohemagglutinin (PHA) and concanavalin A (Con A), demonstrating a marked T-cell deficiency in these populations (15). The response of these cells to lipopolysaccharide (LPS) is intact suggesting normal B-cell function (16). Similar selective deletion of T-cell responses was demonstrated in cultures of all T cell-deficient populations used in the MLR's described below. Table I also shows that AKR and DBA spleen cells treated with 50 μg/ml of mitomycin C (AKR_m and DBA_m) have markedly reduced DNA synthetic responses, but are nevertheless capable of incorporating some [^{3}H]-thymidine ([^{3}H]TdR) in response to the powerful T-cell mitogens, PHA and Con A, confirming reports of incomplete inactivation by mitomycin C (7, 18). AKR and DBA spleen cells treated with 2,000 R irradiation (AKR_x and DBA_x) are virtually incapable of DNA synthesis even in response to mitogens.

TABLE I

Mitogen Responses of Normal, T Cell-Depleted, Mitomycin-Treated, and Irradiated Mouse Spleen Cells

Exp. no.	Cells*	No stimulant	[^{3}H]TdR incorporation		
			PHA	Con A	LPS
			mean cpm + SEM		
1	AKR	1,000 ± 200	50,600 ± 1,400	91,600 ± 5,300	21,600 ± 400
	AKR^{-T}	1,700 ± 200	1,200 ± 100	1,400 ± 50	17,900 ± 2,300
	AKR_m	300 ± 20	8,200 ± 200	17,300 ± 100	500 ± 20
	AKR_x	300 ± 40	200 ± 50	600 ± 50	200 ± 30
2	DBA	1,500 ± 300	40,400 ± 1,600	79,500 ± 9,100	16,700 ± 1,900
	DBA_m	400 ± 60	9,600 ± 500	19,600 ± 1,600	900 ± 60
	DBA_x	200 ± 20	300 ± 50	400 ± 80	140 ± 30

* Spleen cells from normal AKR mice and from thymectomized, irradiated, bone marrow-reconstituted AKR mice treated with burro antimouse thymocyte antiserum 3 and 2 days before sacrifice (AKR^{-T}) were cultured at 4×10^5 cells/0.2 ml culture with nothing, PHA (1 μg/ml), Con A (2 μg/ml), or LPS (10 μg/ml), and [^{3}H]TdR incorporation was determined after 72 h. Some cells were treated with mitomycin C (50 μg/ml) for 30 min at 37°C (m) or exposed to 2,000 R X irradiation at 200 kVCP, half-value layer 0.9 (x), and then washed four times before culture.

Two representative experiments comparing normal AKR and AKR^{-T} spleen cells as responders in the MLR are presented in Table II *a*. A positive DNA

TABLE II

MLR's Involving T-Depleted and F_1 Responder Cell Populations

	Responding cell	Exp. 1		Exp. 2	
(*a*) Response of T cell-depleted populations	AKR	AKR_m*	3,000 ± 200	AKR_x*	5,800 ± 700
		DBA_m	17,800 ± 1,600	DBA_x	16,500 ± 1,700
		F_{1m}	12,800 ± 1,300	F_{1x}	13,200 ± 1,100
	AKR^{-T}‡	AKR_m	4,600 ± 500	AKR_x	3,400 ± 300
		DBA_m	26,000 ± 900	DBA_x	7,400 ± 200
		F_{1m}	5,100 ± 300	F_{1x}	2,900 ± 200
		Exp. 3		Exp. 4	
(*b*) Response of F_1 cell populations	F_1	F_{1m}	6,700 ± 600	F_{1x}	5,000 ± 500
		DBA_m	17,700 ± 1,000	DNA_x	10,000 ± 900
		AKR_m	12,700 ± 800	AKR_x	8,700 ± 600
		AKR_m^{-T}	6,700 ± 600	AKR_x^{-T}	4,800 ± 900

* Target cells were incubated with mitomycin C (50 μg/ml) for 30 min at 37°C (m) or exposed to 2,000 X irradiation (x) and then washed 4 times before culture.

‡ AKR^{-T}: Spleen cells from thymectomized, irradiated, bone marrow-reconstituted mice treated with burro antimouse thymocyte antisera 3 and 2 days before sacrifice.

synthetic response, indicated by incorporation of [^{3}H]TdR in excess of that in syngeneic AKR + $AKR_{m\ or\ x}$ control cultures, occurs when normal AKR spleen cells are mixed with $DBA_{m\ or\ x}$ or $F_{1_{m\ or\ x}}$ target cells. A positive response is also seen when AKR^{-T} spleen cells are mixed with $DBA_{m\ or\ x}$ target cells but no response occurs when AKR^{-T} cells are mixed with $F_{1_{m\ or\ x}}$ target cells. Thus:

$$AKR^{-T} + DBA_{m\ or\ x} \rightarrow \text{response}; \quad AKR^{-T} + F_{1_{m\ or\ x}} \rightarrow \text{no response.}$$

Since F_1 spleen cells should possess essentially all the DBA alloantigens present on parental DBA cells and are able to stimulate MLR's by normal AKR cells, the failure of F_1 cells to stimulate AKR^{-T} cells, which "respond" to DBA cells, is more logically ascribed to an inability of the F_1 "target" cells to recognize AKR antigens than to their failure to present DBA antigens to AKR^{-T} cells. This suggests that the increased DNA synthesis observed when AKR^{-T} cells are mixed with DBA_m or DBA_x cells represents a recognition of AKR antigens by the "inactivated" DBA target cells rather than responsiveness on the part of the T cell-depleted AKR population.

The contention that target cells may, under certain circumstances, be responsible for the immunologic recognition that eventuates in a proliferative response is further strengthened by the studies reported in Table II *b*. In these experiments, F_1 spleen cells are cultured as responder cells with parental DBA or AKR target cells. Mixtures of F_1 cells with inactivated but otherwise normal AKR or DBA parental target cells incorporate substantially more [^{3}H]TdR than do syngeneic control F_1 + $F_{1_{m\ or\ x}}$ mixtures, confirming a previous report

that F_1 cells cultured with mitomycin-treated parental cells yield a positive MLR (9). However, no response is seen when F_1 cells are cultured with AKR target cells depleted of T lymphocytes, although we have previously demonstrated that such AKR^{-T} cells are fully capable of stimulating allogeneic responder cells in the MLR (14). Thus:

$$F_1 + AKR_{m\ or\ x} \rightarrow \text{response}; \quad F_1 + AKR^{-T}{}_{m\ or\ x} \rightarrow \text{no response.}$$

The striking finding in these studies of F_1 hybrid responder cells is that proliferative responses are obtained only when the inactivated parental target cell population contains T cells. This suggests that in mixtures of F_1 cells with $AKR_{m\ or\ x}$ cells, or with $DBA_{m\ or\ x}$ cells, the proliferative response observed is due not to recognition of the inactivated parental cells by the F_1 cells, but to recognition of the F_1 responder cells by the inactivated AKR or DBA target cells.

The data in Table II may be summarized as follows: (*a*) Mixed lymphocyte responses occur when T cell-deficient populations are cultured with allogeneic but not F_1 target cells, while normal spleen cells yield responses when cultured with either allogeneic or F_1 target cells. (*b*) Mixed lymphocyte responses are observed when F_1 cells are cultured with inactivated but otherwise intact parental target cells, but not when F_1 cells are cultured with T cell-deficient parental targets. These essentially symmetrical experiments lead to one of two sets of mutually exclusive conclusions: (*i*) T cells are not required for responsiveness in the MLR but are required as stimulators, and F_1 cells efficiently recognize parental cells but do not present antigens of one parental genotype to responder cells of the other parental genotype; or (*ii*) neither T cell-deficient populations nor F_1 cells respond in the combinations studied, but potentially responsive cells in the inactivated target cell population initiate the reaction leading to increased DNA synthesis in the cultures.

As conclusions *i* are strikingly refuted by recent evidence that T cell-depleted populations are efficient stimulators of normal allogeneic lymphocytes (14, 19) and considerable evidence that F_1 lymphocytes are excellent target cells, not only in MLR's (5, 6), but also in graft-vs.-host responses (20), this set of conclusions appears untenable and leads us to support conclusions *ii* as the proper explanation for the data presented here.

Thus we suggest that in conventional MLR's, responsiveness is dependent upon the presence of T lymphocytes and that F_1 cells do not respond to parental antigens (such as receptor sites) to an extent sufficient to be detected in this system. The capacity of inactivated target cells to initiate responses may well explain the observation that spleen cells from both thymus-deprived and congenitally athymic (*nu/nu*) mice respond to mitomycin C-treated allogeneic cells (8), and, in addition, the previous report of F_1 responsiveness to mitomycin C-treated or irradiated parental cells (9).

The recognition of responder cells by target cells could be translated into

DNA synthesis in two ways. (*a*) The target cell may itself synthesize DNA. This is possible for mitomycin C-treated cells since they are capable of some DNA synthesis in response to T-cell mitogens. However, it is very unlikely that responses seen with X-irradiated target cells can be accounted for in this way, since 2,000 R X irradiation renders these cells incapable of significant DNA synthesis even in response to mitogens. (*b*) The target cell, as a consequence of its recognition of the responder cell, may provoke DNA synthesis in the unpoisoned responder cell, possibly through secretion of a blastogenic mediator. Elaboration of blastogenic factors as a consequence of antigenic recognition has been demonstrated in several systems and does not require DNA synthesis (21–23). Our results indicate that one-way MLR's are best done using parent-F_1 combinations, or alternatively, employing target lymphocyte populations devoid of T cells.

SUMMARY

Mixed lymphocyte reactions occur when mouse spleen cell populations depleted of thymus-derived (T) lymphocytes are cultured with allogeneic target cells inactivated by mitomycin C or X irradiation, and when F_1 hybrid responder cells are cultured with inactivated parental target cells. These responses might be interpreted as indicating that T lymphocytes are not required for responsiveness and that F_1 lymphocytes recognize parental alloantigens. Data reported here indicate that the more likely explanation for these surprising results is that inactivated target cells recognize the "responding" cells and this recognition leads to the response observed.

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