STIMULUS-RESPONSE IN THE MIXED LYMPHOCYTE REACTION

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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 F1 hybrid cells has been used as a natural one-way reaction, since the F1 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) F1 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 F1 cells to parental cells runs counter to the conventional view that F1 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-2k), DBA/2 (H-2d), and (AKR x DBA/2)F1 (F1) mice were obtained from Jackson Laboratory, Bar Harbor, Me. 6-10-wk old AKR mice were thymectomized, irradiated (700 R), and reconstituted with 7 x 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 10^5 mitomycin-treated or irradiated stimulator cells in 0.2 ml of RPMI 1640 supple-
mented with antibiotics and 5% fetal calf serum in round-bottom microtiter wells. Cultures were maintained at 37°C in a humidified 5% CO₂ atmosphere for 72 h. 16 h after addition of 1 µCi of [³H]thymidine ([³H]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⁻⁰) 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 (AKRx and DBAx) have markedly reduced DNA synthetic responses, but are nevertheless capable of incorporating some [³H]-thymidine ([³H]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 (AKRx and DBAx) are virtually incapable of DNA synthesis even in response to mitogens.

Two representative experiments comparing normal AKR and AKR⁻⁰ spleen cells as responders in the MLR are presented in Table II a. A positive DNA
**TABLE II**

**MLR's Involving T-Depleted and F1 Responder Cell Populations**

| Responding cell | Exp. 1                        | Exp. 2                        |
|-----------------|-------------------------------|-------------------------------|
| (a) Response of T cell-depleted populations |                               |                               |
| AKR             | AKR<sup>*</sup> <br>3,000 ± 200 | AKR<sup>*</sup> <br>5,800 ± 700 |
|                 | DBAm <br>17,800 ± 1,600        | DBAm <br>16,500 ± 1,700        |
| F<sub>1m</sub>  | 12,800 ± 1,300                 | F<sub>1x</sub> <br>13,200 ± 1,100 |
| AKR<sup>-T<sup>†</sup> | 4,600 ± 500                   | AKR<sup>x</sup> <br>3,400 ± 300 |
| DBAm<sup>-T</sup> | 26,000 ± 900                   | DBAm<sup>x</sup> <br>7,400 ± 200 |
| F<sub>1m</sub>  | 5,100 ± 300                    | F<sub>1x</sub> <br>2,900 ± 200  |

| Exp. 3 | Exp. 4 |
|--------|--------|
| F<sub>1m</sub> | 6,700 ± 600 |
| DBAm | 17,700 ± 1,000 |
| AKR<sub>m</sub> | 12,700 ± 800 |
| AKR<sup>-T<sup>‡</sup> | 6,700 ± 600 |

| F<sub>1x</sub> | 5,000 ± 500 |
| DNA<sub>x</sub> | 10,000 ± 900 |
| AKR<sup>x</sup> | 8,700 ± 600 |
| AKR<sup>-T<sup>‡</sup> | 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<sup>-T</sup>: Spleen cells from thymectomized, irradiated, bone marrow-reconstituted mice treated with burro antimouse thymocyte antisera 3 and 2 days before sacrifice.

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**synthetic response**, indicated by incorporation of [H]TdT in excess of that in syngeneic AKR + AKR<sub>m or x</sub> control cultures, occurs when normal AKR spleen cells are mixed with DBA<sub>m or x</sub> or F<sub>1m or x</sub> target cells. A positive response is also seen when AKR<sup>-T</sup> spleen cells are mixed with DBA<sub>m or x</sub> target cells but no response occurs when AKR<sup>-T</sup> cells are mixed with F<sub>1m or x</sub> target cells. Thus:

AKR<sup>-T</sup> + DBA<sub>m or x</sub> → response; AKR<sup>-T</sup> + F<sub>1m or x</sub> → no response.

Since F<sub>1</sub> 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<sub>1</sub> cells to stimulate AKR<sup>-T</sup> cells, which “respond” to DBA cells, is more logically ascribed to an inability of the F<sub>1</sub> “target” cells to recognize AKR antigens than to their failure to present DBA antigens to AKR<sup>-T</sup> cells. This suggests that the increased DNA synthesis observed when AKR<sup>-T</sup> cells are mixed with DBA<sub>m or x</sub> 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<sub>1</sub> spleen cells are cultured as responder cells with parental DBA or AKR target cells. Mixtures of F<sub>1</sub> cells with inactivated but otherwise normal AKR or DBA parental target cells incorporate substantially more [H]TdT than do syngeneic control F<sub>1</sub> + F<sub>1m or x</sub> mixtures, confirming a previous report.
that F₁ cells cultured with mitomycin-treated parental cells yield a positive MLR (9). However, no response is seen when F₁ 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:

\[ F₁ + AKR⁻T \rightarrow \text{no response}; \ F₁ + AKR⁻T \text{ or } x \rightarrow \text{no response}. \]

The striking finding in these studies of F₁ 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 F₁ cells with AKR⁻T cells, or with DBAm or x cells, the proliferative response observed is due not to recognition of the inactivated parental cells by the F₁ cells, but to recognition of the F₁ 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 F₁ target cells, while normal spleen cells yield responses when cultured with either allogeneic or F₁ target cells. (b) Mixed lymphocyte responses are observed when F₁ cells are cultured with inactivated but otherwise intact parental target cells, but not when F₁ 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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-F1 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 F1 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 F1 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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