MIXED LYMPHOCYTE REACTIVITY AGAINST NORMAL CELLS BY SPLENIC LYMPHOCYTES FROM TUMOR-BEARING MICE

II. Studies of Autoimmune-Like Activity in Completely Syngeneic and Semisyngeneic Systems

By R. G. DEVLIN, J. D. McCURDY, and P. E. BARONOWSKY

(From the Biochemistry Department, Mead Johnson Research Center, Evansville, Indiana 47721)

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Mice injected with syngeneic or semisyngeneic tumor cell lines eventually succumb to the tumor despite the presence of tumor specific antigens (TSA) on most tumors which have been examined (1). Attempts to explain this phenomenon have usually employed hard experimental evidence which has detected the existence of blocking antibodies (1), soluble TSA (2), and complexes of TSA and blocking antibodies (3), all of which result in the eliciting of weak immune responses against tumor cells. Recently an additional explanation has been suggested (4, 5) which postulates that the induction of an antilymphocytic autoimmune process by tumor cells would seriously impair immunosurveillance capabilities. In the first paper of this series, we reported the finding of vigorous immune reactivity by immunocompetent cells from tumor-bearing F_1 mice against normal parental cells of the strain from which the tumor was derived.

The purpose of the present work was twofold: (*a*) to study autoimmune-like processes of this type in a completely syngeneic system, and (*b*) to examine the strength and the nature of the cellular immune reactions provoked or produced by syngeneic or semisyngeneic mouse tumor cells.

Materials and Methods

Mice.—Mice of the inbred strains DBA/2 (*H-2*^{d}) and BDF_1, which is the hybrid of DBA/2 and C57B1/6 (*H-2*^{b}), were obtained from Jackson Laboratories, Bar Harbor, Maine.

Tumor Cell Line.—L1210 leukemia was routinely maintained in BDF_1 mice as previously described (5) and was transferred to DBA/2 mice in some of these experiments. The L1210 leukemia is of DBA/2 origin.

Mixed Lymphocyte Reactions (MLR's).—Cultures were set up and results compiled as previously described (5).

RESULTS

Spleen cells were collected from DBA/2 mice which had been inoculated with L1210 leukemia cells 7 days earlier (DBAt). When DBAt cells were cultured with normal DBA/2 cells in a two-way MLR, a vigorous immune reaction occurred (Table I, experiment 33). In order to determine the cell type responsible for this activity, DBA/2 or DBAt cells were pretreated with mito-

mycin C before culture (Table I, experiments 34 and 43). Normal DBA/2 cells reacted weakly or not at all to mitomycin-treated DBAt cells. On the other hand, significant immune reactivity by DBAt cells against mitomycin-treated normal DBA/2 cells was noted. This suggestive antiself reactivity occurs only in the DBAt spleen cell population since normal DBA/2 cells do not respond to syngeneic DBA/2 cells (Table II, experiment 27). It is apparent that this activity is at least induced if not produced by the tumor cells.

Ascitic cells from L1210-bearing mice were also found to be capable of responding significantly to normal DBA/2 cells (Table II, experiments 29 and

TABLE I

Mixed Lymphocyte Reactions of DBA/2 Spleen Cells from L1210-Bearing Mice and Normal Syngeneic DBA/2 Spleen Cells

Experiment no.	DPM					Combination	R§
	DBA/2	DBA/2m*	DBAt‡	DBAtm*,‡	MLR		
33	1750		7687		174,996	DBA/2 × DBAt	18.5
	2790		4563		122,332	DBA/2 × DBAt	16.6
34	2790			478	3,478	DBA/2 × DBAtm	1.1
		450	4563		16,906	DBAt × DBA/2m	3.4
	3280		6455		78,090	DBA/2 × DBAt	8.0
	3280			2033	11,547	DBA/2 × DBAtm	2.2
43		588	6455		54,888	DBA/t × DBA/2m	7.8
		588		2033	4,351	DBAtm × DBA/2m	1.6

* Mitomycin-treated spleen cells.
‡ Spleen cells from L1210-bearing DBA/2 mice.
§ DPM in mixed cultures divided by sum of DPM in cultures of one cell type alone.

TABLE II

Mixed Lymphocyte Reactions of Normal and Malignant Cells

Experiment no.	DPM							Combination	R‖
	DBA	DBAm*	BDF	BDFt‡	L1210§	L1210m*	MLR		
24¶			3638			45,702	11,368	BDF × L1210m	<1
36			768			4,622	3,313	BDF × L1210m	<1
24-1				127,390		45,702	311,332	BDFt × L1210m	1.8
28				5,934		6,941	37,653	BDFt × L1210m	2.9
24-2	6,324					45,702	220,057	DBA vs. L1210m	4.2
27	1,722					38,541	73,888	DBA × L1210m	1.8
27	1,722	1,150					3,058	DBA × DBAm	1.1
29		1,064			253,020		559,507	L1210 vs. DBAm	2.2
36		618			97,274		383,576	L1210 vs. DBAm	3.9

* Mitomycin-treated cells.
‡ Spleen cells from L1210-bearing BDF_1 mice.
§ Ascitic L1210 cells from BDF_1 mice.
‖ DPM in mixed cultures divided by the sum of DPM in cultures of one cell type alone.
¶ Experiments no. 24, 24-1, and 24-2 were all done at the same time using the same pools of cells.

36). Both the spleen cell population and the ascitic cell population have been reported to consist almost entirely of malignant cells (6). However, since there are some normal cells present in each case, it is not possible to determine as yet which cell type (normal or malignant) is reacting against the normal syngeneic cells. It is also important to note that there are differences, both morphological (6) and physiological, between splenic L1210 cells and ascitic L1210 cells. The ascitic L1210 cells usually take up much more [^{3}H]thymidine when cultured alone than do splenic L1210 cells. Also, spleen cells from tumor-bearing F_1 mice (BDFt) were able to respond significantly in MLR to ascitic L1210 cells (Table II, experiments 24-1 and 28). One possible explanation for the latter phenomenon might be that it is the few remaining normal cells in the BDFt spleen cell population which are responding to the presumed TSA on the ascitic L1210 cells. However, this may not be the case since normal BDF_1 cells do not respond significantly to ascitic L1210 cells (Table II, experiments 24 and 36). Normal DBA/2 spleen cells, on the other hand, do respond significantly to mitomycin-treated L1210 cells (Table II, experiments 24-2 and 27). Another immunologic difference is also evident between splenic and ascitic L1210 cells. Normal BDF_1 mice, which did not respond to L1210m (Table II), did respond significantly to mitomycin-treated splenic L1210 cells, i.e., BDFtm (Table III, experiments 27, 29 and 32). Also interesting in this regard was the finding that BDFt cells, which responded very vigorously to mitomycin-treated DBA/2 cells (5), did not respond at all to normal mitomycin-treated BDF_1 cells (Table III). Possibly, the presence of C57B1/6 antigens on BDF_1 cells interferes with the recognition of the DBA/2 antigens also present on the F_1 cells. Alternatively, antigen density on the membranes of the F_1 cells may be limiting in this case (7).

DISCUSSION

The data reported in this paper, in which spleen cells obtained from mice-bearing syngeneic tumors react against normal syngeneic cells, do not neces-

TABLE III

Mixed Lymphocyte Reactions of BDFt and Normal BDF_1 Cells

Experiment no.	DPM					Combination	R§
	BDF	BDFm*	BDFt‡	BDFtm	MLR		
27‖	995		7,136		23,571	BDF × BDFt	2.9
27-1	995			3,928	10,526	BDF × BDFtm	2.1
29	2,800			3,058	19,396	BDF × BDFtm	3.3
32¶	1,794			3,727	9,695	BDF × BDFtm	1.8
32-1		465	21,963		13,837	BDFt × BDFm	<1
36		213	5,468		5,076	BDFt × BDFm	<1
32-2	1,794				3,676	BDF × BDF	1.0

* Mitomycin-treated cells.

‡ Spleen cells from L1210 bearing mice.

§ DPM in mixed cultures divided by sum of DPM in cultures of one cell type alone.

‖ Experiments 27 and 27-1 were done at the same time using the same pools of cells.

¶ Experiments 32, 32-1, and 32-2 were done at the same time using the same pools of cells.

sarily imply that an autoimmune reaction is occurring in the tumor-bearing mice. Only the reaction of DBAt cells against autologous lymphocytes would constitute a definite autoimmune process. For this reason, we chose to refer to the present data as representing an autoimmune-like process, which strongly suggests that spleen cells from tumor-bearing mice are able to induce cellular antilymphocytic autoimmunity in host animals. Definitive proof of this suggestion might provide another interpretation for the failure of the immunosurveillance mechanism in mice which succumb to lymphocytic tumors. It might also, hopefully, provide impetus for attempting to find a similar autoimmune reactivity in human cancer patients. Such phenomena have been reported in patients with Hodgkin's disease (8), some of whom at least develop antilymphocytic cytotoxic antibodies. However, if cellular immune activity of this sort is confined to splenic lymphocytes, detection of such activity would involve serious technical difficulties with human patients. Repetition of the experiments reported in this paper on human subjects would require the use of splenic lymphocytes from human patients who are identical twins, or at least identically typed siblings, one of whom had a malignant lymphocytic disease.

The reaction of DBAt cells against normal DBA/2 cells demonstrates that lymphocytes capable of reacting to self determinants do exist. It is not clear whether the reactive lymphocytes are normal or malignant cells. Burnet (9) in his clonal selection hypothesis suggests that all immunocytes capable of reacting to self are destroyed. It is known, however, that acquired tolerance to an antigen can be broken by the use of cross-reacting antigens (10) or by chemically altered antigens, i.e., by adding certain haptenic groups to the protein carrier (11–13). These results might offer an explanation for our findings if we make several assumptions: (*a*) that it is the few remaining normal cells present in the DBAt spleens which are causing the reaction against syngeneic cells and (*b*) that TSA on the malignant cells act as haptens and the normal histocompatibility antigens act as carriers, i.e., the normal cells in the tumor-bearing spleens, during their reactions against the malignant cells (hapten + carrier) would be induced into reacting against normal DBA/2 cells (carrier). However, it is not known if tolerance in cellular immune systems can be broken in the same manner as it is in the humoral systems alluded to above. Such a mechanism would not be surprising, however, in view of the fact that delayed hypersensitivity reactions are carrier specific (14–17).

Interpretation of results dealing with the immunobiology of transplantable tumors is fraught with danger, since some of these tumor cell lines are known to undergo remarkable changes during passage (18). For this reason and for other reasons which will become apparent below, there may be at least three alternative explanations for our findings: (*a*) perhaps the phenomena of "antigenic simplification" or "antigenic modulation" are active in the system studied here. Malignant cells which have been passaged in vivo are known to undergo the process of antigenic simplification, i.e., tumor sublines are established which

are no longer restricted to a specific strain, presumably because they have lost some or all of their *H-2* antigens (18, 19). Also, it has been demonstrated that the exposure of living cells to antibodies against cell surface antigens may lead to the specific disappearance of these antigens (20) by the process of pinocytosis (21), i.e., antigenic modulation. The possibility exists, therefore, that the L1210 tumor cell line used in these experiments had lost its original $H\text{-}2^d$ antigens. This loss, if accompanied by appropriate karyotypic changes, might enable the DBAt cells or the ascitic L1210 cells to recognize the $H\text{-}2^d$ antigens on normal DBA/2 cells as foreign, and thus cause the response seen in the present experiments. A similar theory was proposed by Tyler to explain the origin and development of lymphoid neoplasms (22). Tyler theorized that malignancy developed when a normal cell lost one or more histocompatibility antigens. All normal cells would then be antigenic to the mutated cell and would therefore provide a constant stimulus for unrestrained growth leading to a lymphoid neoplasm. The L1210 cells used in the present experiments have been passaged in this laboratory for 3 yr in BDF_1 mice, and it is not known what effects this passage may have had on the expression of the histocompatibility antigens or genomes of the L1210 cells. However, it must be pointed out that "antigenic simplification" does not seem to have occurred in our L1210 line. It will only grow in BDF_1 or DBA/2 animals and is rejected when transferred to either C57B1/6 or CBA/J mice (unpublished observations). (*b*) Another possible explanation for our data is suggested by experiments in which oncogenic viruses were found to be activated during graft vs. host (GVH) reactions (23). Viruses also appeared in MLR of parental and F_1 cells but not when either lymphocyte population was incubated by itself (24). However, when lymphocyte transformation was blocked by treatment of parental cells with mitomycin C, virus activation did not occur. Perhaps in the system studied here oncogenic viruses are liberated and lead to increased uptake of [^{3}H]thymidine by the reacting cells. However, since in this system and the one reported earlier (5) the normal DBA/2 cells were pretreated with mitomycin C, and since the reacting cells might be malignant cells, such an explanation may not be valid. Also, the presence of viruses is known to inhibit MLR's (25). (*c*) It has recently been shown that thymocytes from certain strains of newborn mice will react significantly in MLR against syngeneic lymphocytes (26). Reactivity is lost in thymocytes from older mice. It could be postulated that DBAt cells or BDFt cells have reverted to a neonatal condition and are thus able to mount an immune reaction against syngeneic cells. It has also been determined that lymphocytes from lymph nodes of rats sensitized in vitro to autochthonous or syngeneic cells were able to cause a GVHR in syngeneic hosts (27). These results suggest that cells capable of reacting against self do exist but are inhibited by factors present in fresh serum (27). The present work, in this paper, indicates that cells capable of reacting to self are either induced or produced by the L1210 leukemia.

One interesting and highly speculative extrapolation of the present work is

that it might suggest an explanation for the peculiar wasting process undergone by animals and human patients with various types of malignancies. On the surface, at least, a striking similarity exists between the cachexia exhibited by some cancer patients and the wasting syndrome of graft vs. host disease (22). In the latter case, the wasting process is the pathological manifestation of GVHR. One could assume that a GVHR is occurring in the DBAt spleen since DBAt cells react in MLR (the in vitro equivalent of GVHR [28]) with normal DBA/2 cells. In this case, however, both the host and the graft would be DBA/2 cells. Since the L1210 injected animals survive for only 7–10 days, it is impossible to determine if they are wasting. The spleens of these animals are always enlarged, but this effect is probably due to increased numbers of tumor cells in the splenic tissue. Interesting in this regard is the finding of a high incidence of malignant lymphomas which occurred in mice undergoing a chronic GVHR (29). Similarly, reovirus 3 has been reported to transform mouse cells which, when injected into newborn animals, cause runting—leading in some cases to lymphomas (30). However, a recent attempt to find a significant autoimmune process in GVHR failed (31). Nor was any significant GVHR evoked by spleen cells from BALB/c mice bearing a testicular tumor when injected into syngeneic recipients (32). On the other hand, the detection of antinuclear antibodies which react with syngeneic nuclei has been reported in mice injected with parental cells (33). The possibility remains, therefore, that some human cancer patients may react similarly to the tumor-bearing mice in these experiments, i.e., some of their immune cells (splenic lymphocytes?) become capable of reacting against self with subsequent impairment of antitumor effectiveness.

SUMMARY

A possible consequence of an antilymphocytic autoimmune process would be serious impairment of an animal's ability to destroy tumor cells. One measure of autoimmune reactivity of this type would be the demonstration of cellular immune responsiveness by cells from tumor-bearing mice against syngeneic normal cells. These experiments demonstrate that spleen cells from mice bearing a lymphocytic leukemia of identical histocompatability type as the host mounted a vigorous immune response against normal syngeneic cells in a mixed lymphocyte reaction (MLR). Moreover, ascitic cells from leukemic mice responded significantly to normal syngeneic spleen cells in MLR's. The former reactions are usually much more vigorous than the responses of normal to malignant cells. These results are discussed in terms of the relationship between autoimmunity and neoplasia. Alternative explanations necessitated by the dangers involved in the interpretation of the immunology of transplantable tumors are considered.

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