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

I. Studies of Vigorous Immune Responsiveness Induced in F_1 Mice by Parental Strain Tumor Cells

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Experimental evidence has been accumulated which suggests a possible relationship between autoimmunity and neoplasia. Approximately 25% of NZB mice, a strain which spontaneously develops lethal autoimmune disease, also develop lymphoid neoplasias (1). Human patients with Hodgkin's disease frequently develop antilymphocyte autoantibodies (2) and about 30% of leukemia patients have detectable antibodies to their own red cells (3). Moreover, autoimmune hemolytic anemia is a frequent complication of chronic lymphocytic leukemia (4) and splenic lymphocytes from mice infected with Rauscher leukemia virus were reported to have mounted an immune response against erythrocytes from syngeneic mice (5). The target cells of autoimmunity in neoplastic diseases are not only limited to lymphocytes and erythrocytes. Cytotoxic effects against normal adult glial cells have been reported in patients with malignant brain tumors (6). Such autoimmune processes might well result in a generalized weakening of the host's immunosurveillance capabilities since it is known that immunosuppressed animals develop neoplasms in greater numbers than normal animals (7).

The establishment of a direct relationship beteen autoimmunity and neoplasia would require the demonstration of an experimentally induced, tumor-dependent autoimmune process. We have studied cellular immune reactions of mice with the aim of detecting possible autoimmune-like reactions associated with the presence of a transplantable L1210 leukemia.

Materials and Methods

Animals.—Female mice of the inbred strains DBA/2 (*H-2*^{d}) and C57B1/6 (*H-2*^{b}) and their F_1 hybrid, BDF_1, were obtained from Jackson Laboratories, Bar Harbor, Maine.

Tumor Cell Line.—L1210 ascitic leukemic cells, which are of DBA/2 origin, were maintained by passage in BDF_1 mice. Recipient mice were injected i.p. with 10^5 L1210 cells in a volume of 0.1 ml. 7–9 days later, ascitic fluid was withdrawn and 10^5 cells were transferred to new recipient animals. Spleen cells from tumor-bearing mice were collected on the 7–9th day after tumor injection.

Mixed Lymphocyte Reactions.—MLR's were performed according to the method of Gazit and Harris (8). Spleen cells were cultured in RPMI 1640 medium supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml) and L-glutamine (200 mM, 2 ml per 100 ml of medium). Mixed lymphocyte cultures contained 3×10^6 cells of each type (a total of 6×10^6 cells) in 3 ml of medium. Control cultures contained 3×10^6 cells from either source, alone, in 3 ml of medium. Three replicates of each cell type or combination were done. After 3 days, 1 ml of RPMI 1640 culture medium containing 1 μCi/ml tritiated thymidine was added to each culture tube. 18–24 h later, cultures were harvested with cold TCA, solubilized in NCS tissue solubilizer (Amersham-Searle, Arlington Heights, Ill.), dissolved in Liquifluor (New England Nuclear Corp., Boston, Mass.), and counted for tritiated thymidine incorporation in a liquid scintillation counter. One-way mixed lymphocyte reactions were performed by treating cells with 25 or 50 μg/ml mitomycin-C for 30 min at 37°C, washing twice, incubating for 10 min at 37°C, and washing again before placing the mitomycin-treated cells in culture.

Results are recorded as DPM incorporated per culture and as the ratio (R) of DPM in mixed cultures divided by the sum of DPM in cultures of each cell type alone. A ratio of approximately 1 indicates that no significant reaction has occurred. A ratio of approximately 2 or more indicates a significant reaction.

RESULTS

Normal DBA/2 spleen cells were cultured with spleen cells from hybrid BDF_1 mice bearing the L1210 tumor (BDFt) in a two-way MLR (experiment 24, Table I). A surprisingly high response ($>10^6$ DPM) resulted, despite the lack of response in cultures of normal DBA/2 cells and normal BDF_1 cells in the same experiment. We hypothesized that the strong response was due primarily to the reaction of the normal DBA/2 cells against the postulated

TABLE I

Mixed Lymphocyte Reactivity of Spleen Cells from Tumor-Bearing F_1 Mice Against Normal Parental Cells

Experiment no.	DPM						Combination	R§
	BDFt*	BDFtm‡	DBA	DBAm‡	BDF	MLR		
24	127,390		6,324			1,132,342	BDFt × DBA	8.5
			6,324		3,638	5,667	BDF × DBA	<1
27	7,136		1,722			503,557	BDFt × DBA	56.8
		3,928	1,722			19,956	DBA × BDFtm	3.5
	7,136			1,150		87,845	BDFt × DBAm	10.6
			1,722		995	6,258	BDF × DBA	2.3
28	5,934		6,901			373,024	BDFt × DBA	29.1
		959	6,901			13,007	DBA × BDFtm	1.7
	5,934			1,371		194,700	BDFt × DBAm	26.7
32	21,963		1,880			743,932	BDFt × DBA	31.2
		3,727	1,880			10,150	DBA × BDFtm	1.8
	21,963			567		492,424	BDFt × DBAm	21.9
33	5,247			194		40,204	BDFt vs. DBAm	7.4

* Spleen cells from L1210-bearing BDF_1 mice.

‡ Mitomycin-treated spleen cells.

§ Counts in mixed cultures divided by sum of counts in cultures of each cell type alone.

tumor-specific antigens (TSA) present on the BDFt cells. This hypothesis was tested by treating BDFt cells or normal DBA/2 cells with mitomycin-C, before culturing them with untreated cells (experiments 27, 28, 32, and 33, Table I). Surprisingly, DBA/2 cells responded relatively weakly against mitomycin-treated BDFt cells, whereas a very strong response was seen in all four experiments where BDFt cells were reacted against mitomycin-treated DBA/2 cells. Control cultures of normal BDF_1 × DBA/2 cells had approximately the same level of response as the DBA/2 × BDFtm cultures, indicating that the *H-2* antigens on the BDFt cells were not different from those on normal BDF_1 cells. It is apparent from these experiments that spleen cells from mice bearing the L1210 leukemic tumor were able to mount a very vigorous immune response against mitomycin-treated normal cells which had no different *H-2* antigens than those on the reacting cells.

The specificity of the response of BDFt cells in this system was examined by reacting the BDFt cells against parental C57B1/6 spleen cells (i.e., the alternate parental strain which does not share *H-2* antigens with the tumor cell line). In experiments 35 and 36 (Table II), it can be seen that BDFt cells failed to react against normal C57B1/6 cells. As expected, however, the C57 B1/6 cells did respond significantly to mitomycin-treated BDFt cells, and BDFt cells responded vigorously to DBAm cells. We concluded that the response reported in Table I was immunologically specific in that it was directed against only normal cells of the same strain as the tumor cells, i.e., DBA/2.

As seen in Table I, BDFt cells responded much more vigorously to normal DBA/2 cells than did normal BDF_1 cells. This comparison may not be valid, however, since cells from a tumor-bearing animal had been exposed previously to tumor cells of DBA/2 origin and were presented with DBA/2 antigens for the second time when they were cultured with DBA/2 spleen cells in a MLR. For this reason, we examined the effects of injecting normal BDF_1 mice with normal DBA/2 spleen cells before culturing them with DBA/2 cells in MLR's (Table III). BDF_1 mice were injected with varying amounts

TABLE II

Mixed Lymphocyte Reactivity of Spleen Cells from Tumor-Bearing F_1 Mice against Normal Parental Cells

Experiment no.	DPM						Combination	R§
	BDFt*	BDFtm‡	C57B1/6	C57B1/6m‡	DBA/2m‡	MLR		
35	18,049		353			15,756	BDFt × C57	<1
		1,339	353			3,382	C57 × BDFtm	1.9
	18,049			178		10,231	BDFt × C57m	<1
36	5,468			149		4,585	BDFt × C57m	<1
	5,468				618	120,056	BDFt × DBAm	20.9

* BDF_1 spleen cells from L1210-bearing BDF_1 mice.

‡ Mitomycin-treated spleen cells.

§ Counts in mixed cultures divided by sum of counts in cultures of each cell type alone.

TABLE III

Mixed Lymphocyte Reactivity of BDF_1 Spleen Cells from Mice Injected with Parental Spleen Cells, against Normal Parental Spleen Cells

DPM						Combination	R
BDF.1*	BDF_{21}‡	BDF_{85}§	BDFt‖	DBAm¶	MLR		
5,499				194	2,084	BDF.1* × DBAm	<1
	2,549			194	2,376	BDF_{21}‡ × DBAm	<1
		2,677		194	3,191	BDF_{85}§ × DBAm	1.1
			5,247	194	40,204	BDFt × DBAm	7.4

* BDF_1 spleen cells 7 days after i.p. injection of 0.1×10^6 DBA/2 spleen cells.
‡ BDF_1 spleen cells 7 days after i.p. injection of 21×10^6 DBA/2 spleen cells.
§ BDF_1 spleen cells 7 days after i.p. injection of 85×10^6 DBA/2 spleen cells.
‖ BDF_1 spleen cells from L1210 bearing mice.
¶ Mitomycin-treated spleen cells.

of normal DBA/2 spleen cells 7 days before harvesting the BDF_1 spleen cells for MLR's. Injection of 0.1×10^6 DBA/2 spleen cells corresponded to the number of L1210 cells normally employed in transferring the tumor. Other mice were injected with 21×10^6 or 85×10^6 DBA/2 spleen cells. It can be seen in Table III that none of the preinjection schedules resulted in a significant MLR when spleen cells from injected animals were reacted against normal DBA/2 cells. The response of BDFt × DBAm in the same experiment was highly significant. It is obvious from these experiments that pre-exposure to DBA/2 antigens was not responsible for the suggestive antiself activity seen in cultures of BDFt × DBAm cells.

DISCUSSION

The initial purpose of these experiments was to determine whether normal spleen cells would react in MLR against semi-syngeneic spleen cells from tumor-bearing mice (BDF_1). The spleens of L1210-bearing mice have been reported to consist almost entirely of malignant leukemic cells 7 days after inoculation (9). If the splenic L1210 cells bore tumor-specific antigens (TSA) on their surfaces, a significant MLR might result when the tumor cells were cultured together with normal spleen cells of the same strain as the tumor cells, i.e., DBA/2. In fact, however, the contrary result was observed: spleen cells from tumor-bearing F_1 mice reacted vigorously to mitomycin-treated, normal parental cells. This response was specific both for the tumor-bearing spleen cells (BDFt) and for the parental cells from the strain from which the tumor was derived (DBA/2). Normal BDF_1 spleen cells reacted only weakly against DBA/2 cells, and BDFt spleen cells reacted only weakly against cells from the other parent (C57B1/6).

The obvious question arising from the experiments reported here is: which cell type, normal or malignant, is responsible for the suggestive antiself reactivity which has been revealed? This important differentiation of reactive cell type might be answered by experiments currently in progress using solid

L1210 tumors induced by s.c. injection of L1210 cells. However, since both normal and malignant cells possess *H-2*^{d} antigens, neither cell type would be expected to react against normal DBA/2 cells which are also *H-2*^{d} antigenically. Thus, the end result would be the same, no matter which type is involved, i.e., a tumor-induced antiself reactivity which presumably would weaken the host's attack against the tumor. A better experiment to illustrate this autoimmune-like process would employ spleen cells from tumor-bearing DBA/2 mice and normal DBA/2 spleen cells in MLR. This type of experiment would eliminate any complications resulting from the use of semisyngeneic cells, as described in this report, by using an entirely syngeneic system. It would also be necessary to determine if the same type of reactions occur using autologous cells. The former experimental system will be described in the next paper of this series, and the results will be discussed in terms of the necessary caution which must be exercised in interpreting experiments concerned with the immunology of transplantable tumors.

SUMMARY

The establishment of an intimate connection between autoimmunity and neoplasia would require the demonstration of an experimentally induced, tumor-dependent autoimmune process. For this reason, we have studied cellular immune reactions of mice bearing a transplantable leukemia (L1210). Spleen cells from hybrid BDF_1 mice bearing the L1210 tumor (BDFt) reacted vigorously in mixed lymphocyte culture with mitomycin-treated, normal spleen cells from mice of the parental strain from which the L1210 tumor was derived (DBA/2). Spleen cells from nontumor-bearing BDF_1 mice reacted only weakly with these parental cells. The BDFt cells likewise did not respond when cultured with mitomycin-treated spleen cells from the other parental strain (C57B1/6). The vigorous mixed lymphocyte reaction (MLR) by BDFt cells against normal parental cells of the same strain as the tumor was not due to a double exposure of the reacting cells to histocompatibility antigens shared by tumor cells and normal parental cells. The response of cells from tumor-bearing F_1 mice against normal parental cells seen in these experiments suggests the possibility of the induction of an autoimmune-like process against host lymphocytes by spleen cells from leukemic mice. Theoretically such a phenomenon would considerably reduce an animal's ability to mount an immune attack against malignant cells.

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