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Cell-Mediated Immunity to Plasma Cell Myeloma: Specific Inhibition by a Serum Factor Adhering to Effector Lymphocytes[1] (38382)

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Plasma cell myeloma usually presents as a primary tumor of the bone marrow and consists of an abnormal clone of plasma cells. The clonal proliferation is usually evident by the production of abnormal homogenous immunoglobulin which may circulate in the plasma. The antigenic and immunogenic properties of myeloma cells and proteins have been recently demonstrated in animal models in terms of immuno-protection against plasma cell tumor growth (1, 2). In the human, the antigenicity of the abnormal immunoglobulins has also been documented in terms of humoral anti-IgG response in the tumor-bearing patients (3). This suggests the presence of myeloma-associated antigen. An interaction between peripheral blood lymphocytes and autologous myeloma cells, if detected *in vitro* by the blastogenic response method (4, 5) would confirm the finding of myeloma-associated antigen and further indicate the presence of myeloma-directed cellular immune reactivity.

Materials and Methods. Ten patients with plasma cell myeloma were studied. Their general immunocompetence was assessed by skin testing for delayed hypersensitivity reaction (DH) to a battery of recall antigens and to a challenge of dinitrochlorobenzene (DNCB) following primary sensitization (6, 7). Bone marrow specimens were aspirated with heparinized syringe from nine patients and in one patient (No. 1) malignant pleural effusion was the source of plasma cells. Separation of plasma cells was achieved by differential flotation on ficoll-hypaque gradient as previously described (8). Briefly, diluted bone marrow suspension or pleural effusion was carefully layered on top of a mixture of 9% ficoll (10 parts) and 33.9% hypaque (24 parts). After centrifugation at 1500*g* for 15 min, the floating fraction of cells was aspirated and contained from 30 to 90% plasma cells contaminated with granulocytes, lymphocytes, and a few red blood cells. This cell fraction was washed twice, counted, and resuspended in minimal essential media (MEM).

Venous blood was drawn on the day of plasma cell procurement. After defibrination with glass beads, and dextran sedimentation of red blood cells, the leukocytes were separated from the serum by centrifugation. Half of the leukocytes were then thoroughly washed six times in MEM, and the other half remained unwashed. Washed leukocytes were divided into two groups: one was resuspended in autologous patients' serum and the other half in pooled normal human serum. Unwashed leukocytes were likewise resuspended in autologous patients' serum and pooled normal human serum. Accordingly, four groups of lymphocyte cultures were set up in 16 × 125 mm screw cap tubes. Each culture contained 10^6 responding lymphocytes, 1 ml of serum, and 2 ml of MEM. In addition to unstimulated controls, duplicate lymphocyte cultures were stimulated with PHA and with 10^6 autologous plasma cells irradiated with 4000 R just before use. The irradiated plasma cells were also cultured alone and with irradiated autologous lymphocytes. After seven days of incubation at 37° in an atmosphere of 5% CO_2 in air, 2

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μCi of tritiated thymidine (specific activity 1.9 Ci/mmole) was added and cultures were harvested 3 hr later as previously described (9). Blastogenesis was interpreted in terms of the incorporation of tritiated thymidine into the acid insoluble fraction measured by liquid scintillation and expressed in counts per minute per 10^6 lymphocytes. The stimulation index (S.I.) was calculated for lymphocyte-plasma cell interaction by subtracting the CPM of the appropriate controls (cultures containing plasma cells alone or mixture of irradiated lymphocytes and plasma cells — whichever was higher) from the CPM of unstimulated lymphocytes. A stimulation index equal to or greater than 3.0 was considered positive blastogenic response to myeloma cells since stimulation indices of less than 3.0 were regularly observed in preliminary studies of cancer patients where peripheral blood lymphocytes were cultured with autologous normal bone marrow cells (10).

Results. Results are shown in Table I. A positive blastogenic response (S. I. > 3.0) to autologous myeloma cells *in vitro* was observed in three of ten cases (patients No. 1, 7, 10). All three patients were immunocompetent as judged from the positive delayed hypersensitivity skin reactions to recall antigens and to DNCB. In two patients (No. 1 and 7) the blastogenic response to myeloma cells occurred only with extensively washed lymphocytes resuspended in pooled normal human serum. Resuspension of these washed lymphocytes in the patients own serum resulted in a complete abrogation of the effect with blastogenic responses similar to those of unwashed lymphocytes (S. I. < 3.0). This abrogation of the blastogenic response among washed lymphocytes by the patients own serum appears to be tumor-specific since no such effect was noted in the response of lymphocytes to PHA. Protein electrophoretic analysis of the first supernatant obtained from the lymphocyte washing in case No. 1 showed a pattern similar to the one found in the serum of this patient with a sharp peak at the gammaglobulin zone (Fig. 1). Although the study of washed lymphocytes was not done in case No. 10, it is noteworthy that positive blastogenic response to autologous myeloma cells has occurred among unwashed lymphocytes only in the presence of normal serum. The response to PHA remained poor in either serum in this case.

Six patients were totally unreactive to their

TABLE I. Lymphocyte Blastogenic Responses to Autologous Myeloma Cells. H^3-Thymidine Incorporation (C.P.M. per 10^6 Lymphocytes).[a]

Patient No.	Disease status	D.H.	Unwashed lymphocytes										Washed lymphocytes									
			Patient's serum					Normal serum					Patient's serum					Normal serum				
			L	L_{PHA}	P_r	$L_r + P_r$	L + P_r S.I.	L	L_{PHA}	P_r	$L_r + P_r$	L + P_r S.I.	L	L_{PHA}	P_r	$L_r + P_r$	L + P_r S.I.	L	L_{PHA}	P_r	$L_r + P_r$	L P_r S.I.
1	Active	+	740	124192	15	21	675 0.8	735	170178	63	37	315 0.3	655	132395	15	33	448 0.6	255	167639	146	37	1375 4.8
2	Active	+	1420	70000	43	62	190 0.08	121	66249	195	66	81 0	2180	78684	50	60	175 0.05	380	92559	160	34	90 0
3	Active	–	370	124396	4854	6337	5050 0	615	69041	3524	5599	4720 0	315	83734	260	863	34 0	52	64413	501	501	356 0
4	Active	–	2885	30926	139	91	2818 0.8	735	24642	78	233	1910 2.4	*	*	*	*	* *	*	*	*	*	* *
5	P.R.	–	3658	57138	39	72	3788 1.0	6594	53094	36	35	6600 0.9	3830	55999	56	133	2550 0.6	5025	74178	49	18	2712 0.5
6	Active	–	3835	7768	1487	1647	1975 0.08	2145	5406	1332	1263	1640 0.1	1185	3404	300	980	1960 0.8	1275	5089	1089	939	1280 0.1
7	P.R.	+	595	29813	27	50	1300 2.1	775	44627	85	49	1585 1.9	210	28273	173	36	472 1.4	300	32955	53	119	1055 3.1
8	C.R.	–	447	14403	189	95	626 0.9	444	17879	7	46	250 0.4	260	5879	25	97	472 0.6	590	16271	29	521	550 0.05
9	Active	+	344	47216	2481	1705	2760 0.8	140	32384	836	1051	1026 0	120	32854	4759	2246	2000 0	41	26937	866	1375	900 0
10	Active	+	212	2332	34	22	275 1.1	275	4177	22	20	1283 4.5	*	*	*	*	* *	*	*	*	*	* *

[a] Not done, P.R. Partial remission, C.R. Complete Remission, D.H. Delayed hypersensitivity, L Unstimulated lymphocytes, L_{PHA} PHA stimulated lymphocytes, P Irradiated plasma cells, $L_r + P_r$ Irradiated lymphocytes + irradiated plasma cells, L + P_r Lymphocytes stimulated with irradiated plasma cells, S.I. Stimulation index (see text for calculation).

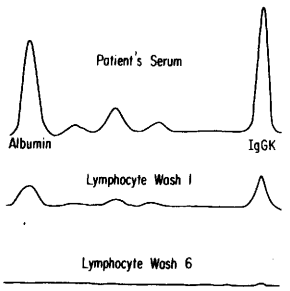

FIG. 1. Protein electrophoretic analysis of serum and supernatants from the lymphocyte washing in case No. 1.

own myeloma cells in any combination of lymphocytes and serum (one nonresponder was not studied after extensive washing). This nonreactivity could be related to multiple factors such as toxicity of plasma cells to the responding lymphocytes (case No. 2), poor general immunocompetence (negative D.H. in cases No. 3, 4, 5, and 6 and poor response to PHA in cases No. 6 and 8), "feeder" layer-like phenomenon between inactivated lymphocytes and plasma cells interfering with the intrepretation of positive blastogenesis to plasma cells (cases No. 3 and 9) and higher base line "control" of lymphocyte activity (cases No. 5 and 6).

Discussion. These results demonstrate the presence of myeloma-associated antigen and the *in vitro* cellular immune response directed against it in some cases. This is in agreement with previous studies demonstrating cell-mediated immune reactivity against other solid tumors and also against leukemic blasts (11–14). The myeloma-associated antigen is either a distinct antigen on the surface of malignant plasma cells, or perhaps it may be related to the abnormal immunoglobulin produced by this tumor as previously reported (3). Our study also suggests that a serum factor in some patients binds to circulating lymphocytes and renders them specifically incapable of reacting with autologous myeloma cells. Upon removal of this adhering substance from the lymphocyte surface by extensive washing, they become reactive to myeloma cells. This is similar to the studies of Currie *et al.* (15) who showed that extensive washing of lymphocytes restored their cytotoxic activity against tumor cells with abrogation of the effect upon resuspension of these lymphocytes in the patients own serum. These investigators suggested that this serum-mediated blocking (at the level of effector lymphocytes) is exerted by circulating antigenic determinants released by the tumor. Baldwin *et al.* have recently demonstrated specific inhibition of lymphocyte cytotoxicity against colon carcinoma cells after incubation of the lymphocytes with "membranes" prepared from these tumor cells (16). Although it is quite possible that a distinct and unidentified myeloma-associated antigen was eluted from the surface of the lymphocytes, the finding of positive blastogenic response among washed lymphocytes to myeloma cells in the presence of normal but not the patients own serum, and the detection of abnormal immunoglobulin in the supernatant obtained from the lymphocyte washing suggest the possibility of a close relationship between the abnormal immunoglobulin and the myeloma-associated antigen. The latter assumption may lead to the following paradoxical situation: the abnormal immunoglobulin produced by the malignant plasma cells may carry some foreign antigenic determinants. Its presence on the surface of plasma cells is responsible for the cellular immune reactions observed *in vitro,* and its release into the circulation may result in its binding to the surface of lymphocytes *in vivo* followed by a specific interference with the aforementioned cellular immune reaction. This phenomenon, however, may be an important parameter in the host-tumor relationship which may allow the tumor cells to proliferate despite their recognition by the host as foreign entity.

Summary. Tumor-directed immune reactivity was studied in ten patients with plasma cell myeloma by the lymphocyte blastogenesis method. Three patients showed positive reactions to their own myeloma cells. In two of them this reactivity was detected only among extensively washed lymphocytes in the presence of normal serum and specifically inhibited by the patients' own serum. A supernatant obtained from the lymphocyte washing in one patient revealed the same abnormal immunoglobulin which circulated in the plasma of this patient. This suggests but does not prove that the abnormal immunoglobulin on the surface of myeloma cells may carry foreign antigenic determinants which can evoke a cellular immune response in the host while its circulating counterpart may specifically block this immune response by binding to the surface of the effector lymphocytes.

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