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Mechanisms by which Tumors Avoid Destruction by the Immune System
BCG-Catalyzed Increase in IgG and IgA Blocking Activity of Lymphocyte-Mediated Cytotoxicity

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Durch welche Mechanismen wird die Zerstörung von Tumoren durch das Immunsystem verhindert? BCG-katalysierter Anstieg von IgG und IgA mit blockierender Aktivität gegenüber lymphozyten-vermittelter Zytotoxizität.

Zusammenfassung. Die Immunglobulinfraktion IgM aus dem Serum von einwöchig-sarkom-tragenden BALB/c Mäusen steigerte die lymphozyten-vermittelte zytotoxische Freisetzung von ^{51}Cr aus ^{51}Cr-markierten Sarkomzellen, während die IgG- und IgA-Fraktionen desgleichen Tieres keinen Effekt hatten. Diese beiden letzteren Immunglobulinfraktionen von Mäusen, welche ein Sarkom 14 Tage oder länger trugen, inhibierten die lymphozyten-vermittelte zytotoxische Aktivität. Die IgA-, IgG- oder IgM-Fraktionen von Mäusen nach Inokulation mit Bazillus Calmette-Guerin (BCG) hatten keine Wirkung; wenn die gleichen Versuchstiere jedoch mit lebenden Sarkomzellen (10^2 bis 10^6 Zellen pro Maus) zusätzlich inokuliert wurden, hatten die IgG- und IgA-Fraktionen eine Hemmwirkung auf die lymphozyten-vermittelte zytotoxische Aktivität. Die Hemmwirkung war am stärksten mit Seren oder IgG von tumortragenden BCG-behandelten Tieren.

Schlüsselwörter: Immunglobuline; lymphocyten-vermittelte Zytotoxität; BCG-Blockierung von lymphozyten-vermittelter Aktivität.

Summary. The immunoglobulin IgM fraction from the serum of one week sarcoma-bearing BALB/c mice increased, the IgG and IgA fractions from the same animal had no effect on the lymphocyte-mediated cytotoxic release of ^{51}Cr from ^{51}Cr-labelled sarcoma cells. These latter two immunoglobulin fractions from serum of 14 Days, or more, sarcoma-bearing mice inhibited the lymphocyte-mediated cytotoxic activity. The IgA, IgG or IgM fractions from mice inoculated with bacillus Calmette-Guerin (BCG) had no effect, but if these same animals were inoculated with viable sarcoma cells (10^2 to 10^6 cells per mouse) IgG and IgA fractions inhibited the lymphocyte-mediated cytotoxic activity. The magnitude of inhibition was greatest with sera or IgG from tumor-bearing BCG-treated animals.

Key words: Immunoglobulins, lymphocyte-mediated cytotoxicity, BCG-blocking of lymphocyte-mediated activities.

Factors which can block tumor-cell destruction in vivo and in vitro by lymphocyte-immune to tumor-associated antigens have been shown in the serum of tumor-bearing patients [1–3]. This is an escape mechanism from lymphocyte cytotoxicity.

There is an increasing evidence which suggests that IgG_2 eluted from chamically induced mouse sarcomas enhance the growth of the respective sarcomas in vivo [4], and that Burkitt lymphoma cells growing in vivo are coated with IgG immunoglobulin [5].

The present studies demonstrate that IgM enhances, while IgG and IgA block the lymphocyte-mediated cytotoxic activity against ^{51}Cr-labelled sarcoma cells. Administration of viable bacillus Calmette and Guerin (BCG) similar to treatment with killed sarcoma cells catalyze the IgA and IgG mechanisms by which the sarcoma cells avoid destruction by lymphocyte-mediated immune responses.

Materials and Methods

Viable bacillus Calmette and Guerin (BCG) was obtained from the BCG-Laboratories of the University of Illinois, Chicago, Illinois, USA. Sarcoma P 1978 was obtained from the tumor collection of the National Institute of Health, Bethesda, Maryland, USA. The sarcoma cells were continuously transferred in BALB/c mice. Seven days after s.c. inoculation of 10^4 viable sarcoma cells into the right hind axle of the mouse, the mice were sacrificed by cervical dislocation, the spleens were removed immediately, rinsed, minced into small pieces, and incubated in essential Eagle's medium (MEM) supplemented with 10% heat inactivated calf serum, penicillin (100 u) -streptomycin (100 ug), collagenase (120 u) and hyaluronidase (50 u). During the 120 min incubation period at 37° the tissue-enzyme mixture was stirred magnetically, and pelleted at 150 g for 5 min. The lymphocytes were washed three times with MEM, adsorbed on Nylon columns and eluted with calf serum. These preparations contained 95 to 99% viable lymphocytes as evaluated by the exclusion test in 0.1% trypan blue. The cytotoxic activity was assessed by the method of Brunner *et al.* [6]. The gamma globulin fractions were prepared by three different techniques: The IgG was obtained from ammonium sulfate precipitation followed by dialysis against 0.005 M phosphate buffer, and elution from DEAE-cellulose columns and dialysis against 1.0% ammonium carbonate. The IgM was isolated from serum treated with 10 parts of 0.75% H_3BO_3, dissolved in 0.1 M Tris-0.2 M NaCl of pH 8.0, and purified on Sephadex G-200. It had an $S_{20,w} = 18.3$ s. The IgA was obtained from serum diluted with 0.9% NaCl, and 0.1 M $ZnSO_4$. The pH adjusted to 6.93 and centrifuged. The supernatant was added to glycine to a final concentration of 2%, and the globulin was precipitated with ammonium sulfate full saturation. The precipitated globulin was dissolved in distilled water and finally desalted on Sephadex G-50. It had an $S_{20,w} = 6.9$ s.

Results

Spleen lymphocytes from 7 days sarcoma-bearing animals have a direct cytotoxic effect against ^{51}Cr-labelled sarcoma cells. Gamma globulins from serum of normal mice had no effect on the lymphocyte-mediated cytolytic release of ^{51}Cr- into the cell-free medium.

Although, IgM fraction from the serum of one-week sarcoma-bearing mice increased, the IgG and IgA fractions had no effect on the lymphocyte-mediated release of ^{51}Cr from ^{51}Cr-labelled sarcoma cells. On the other hand, IgG and IgA fractions from serum of 14 Days, or more, sarcoma-bearing mice inhibited, the IgM fraction had no effect on the lymphocyte-mediated cytotoxic activity. These experiments suggest changes in the activity of the serum globulin fractions during progressive growth of the sarcoma.

Although, the IgM fraction from the serum of mice treated with killed sarcoma cells enhanced, the IgA and IgG fractions had no effect on the lymphocyte-mediated cytotoxic activity. If these animals were challanged with 10^4 viable sarcoma cells, the IgM fraction looses its enhancing effects, while the IgA and IgG fractions inhibit the lymphocyte-mediated cytotoxic activity.

The IgA, IgG and IgM from the serum of BCG-treated mice had no effect, but if these animals were challanged with 10^4 viable sarcoma cells, the IgA and IgG fractions inhibit the lymphocyte-mediated cytolytic release of ^{51}Cr from ^{51}Cr-labelled sarcoma cells. These experiments indicate that if administered into mice treated with either killed sarcoma cells or with BCG, viable sarcoma cells produce IgA and IgG fractions which protect the sarcoma cells from destruction by the lymphocyte-mediated immune systems.

The data summarized in the table, also indicate that the IgA and IgG fractions from the serum of animals treated with incubation mixtures containing BCG with spleen lymphocytes from tumor-bearing animals inhibit, but BCG with spleen lymphocytes from normal animals had no effect on the lymphocyte-mediated cytolytic destruction of the sarcoma cells.

Conclusion

These experiments suggest that BCG functions through lymphocyte-mediated mechanisms which regulate the action of gamma globulins. At least two qualitatively different activities are reported, one concentrated in the IgM fraction leading to enhancing, the other concentrated in the IgA and IgG fractions inhibiting the lymphocyte-cytolytic damage of the sarcoma cells. Both IgA and IgG protect the sarcoma cells from the lymphocyte-mediated cytolytic activities.

Table 1. Globulin "Enhancing" or "Blocking" Destruction of the Sarcoma Cells by Lymphocytes-Mediated Immune Systems

Spleen lymphocytes from seven days tumor-bearing animals (10^7 viable cells) were incubated with ^{51}Cr-labelled sarcoma P1798 (10^7) viable cells in presence of 5 ug of the indicated globulin fraction in a total volume of 1.5 ml of MEM supplemented with 10% heat inactivated calf serum. Incubation at 37° in a moist 5% CO_2 air atmosphere for 30 min. The results have been corrected for the activity released spontaneously, and represent the average of five separate assays ± SD. – The activity released spontaneously 317 ± 17 Cpm ^{51}Cr counts per min. incorporated into the target cells 7961 ± 381 Cpm/10^5 viable sarcoma cells.

Serum from Mice	Globulin	Radioactivity CPM	Released %
MEM		4357 ± 211	60 – 69
Normal	IgG	4343 ± 208	61 – 70
	IgM	4486 ± 212	63 – 76
	IgA	4272 ± 201	60 – 67
Tumor-Bearing (1st Week)	IgG	4699 ± 233	66 – 71
	IgM	5838 ± 263	82 – 89
	IgA	4201 ± 208	59 – 64
Tumor-Bearing (2nd Week)	IgG	1495 ± 75	21 – 26
	IgM	4770 ± 215	67 – 72
	IgA	2350 ± 113	33 – 39
Pretreated with killed sarcoma cells	IgG	4210 ± 210	59 – 64
	IgM	6693 ± 296	94 – 98
	IgA	4349 ± 214	61 – 66
Pretreated with killed sarcoma cells, then Challenged with Viable sarcoma cells	IgG	1495 ± 75	21 – 25
	IgM	4198 ± 206	59 – 63
	IgA	1210 ± 64	17 – 22
Pretreated with BCG (4.5 mg/mouse)	IgG	3702 ± 142	52 – 57
	IgM	4268 ± 213	60 – 64
	IgA	4550 ± 221	64 – 69
Pretreated with BCG (4.5 mg/mouse), then Challenged with Viable sarcoma cells	IgG	2919 ± 168	41 – 46
	IgM	4404 ± 220	62 – 67
	IgA	1994 ± 96	28 – 32
Pretreated with preincubated BCG-Spleen lymphocytes from sarcoma-bearing mice	IgG	362 ± 17	8 – 13
	IgM	4269 ± 208	60 – 64
	IgA	783 ± 311	11 – 17
Pretreated with preincubated BCG-Normal lymphocytes	IgG	4127 ± 215	58 – 61
	IgM	5621 ± 262	72 – 77
	IgA	4473 ± 223	63 – 67

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