Bulgarian Academy of Sciences

Acta cytobiologica et morphologica, 2
Sofia • 1992

Immune response inhibition in mice bearing sarcoma 180 Crocker, plasmocytoma Sp-2/0 Ag 14 or hybridoma

Tz. Mihova, D. Nikolova, I. Goranov*, M. Bratanov**

Department of Pathology, Medical Academy, Sofia
** Institute of Cell Biology and Morphology, Bulgarian Academy of Sciences, Sofia*

The cells of the immune system play a definite surveilance role against neoplastic diseases. There is no reason to dispute that immunological response influences the course of tumour growth and may be manipulated to benefit the host [1].

The purpose of the present study is to investigate the influence of three experimental tumours – sarcoma 180 Crocker, plasmocytoma Sp-2/0 Ag 14 and hybridoma, secreting monoclonal antibodies against the hapten dinitrophenilic group, on the immune response in mice.

Key words: immune response, sarcoma 180 Crocker (Sa 180), plasmocytoma Sp-2/0 Ag 14 (Pl), hybridoma, (H), plaque-forming cells (PFC).

Material and methods

Animals: 150 inbred, 2 to 3 months old BaLb/c mice of both sexes.
Experimental scheme: One group of mice (70) has been investigated on the 12th day and the other group of mice (70) on the 30th day after tumour-cell inoculation. Each of these groups have been subdivided into three subgroups of 20 and one of 10 mice for control. The control group has been injected with saline, while the other three groups have been inoculated subcutaneously with a suspension of 10^6 tumour cells in 0,1 ml from each one of the tumours. The vitality of tumour cells has been checked by the test of exclusion of dead cells with tripan blue. A suspension of 96% vitality of tumour cells has been applied for transplantation.

The number of antibody synthesising cells has been established by Dresser and Greaves [3].

The delayed type hypersensitivity (iduced by dinitrophenolbenzol – DNFB) has been determined by method of Cluman, modified by Bratanov et al. [2].

Results

The immune response to sheep red blood cells (SRBC) established by the quantity of PFC in the spleen shows significant variations in the three types of tumours depending on the presence of palpable tumour growth (Fig. 1). On the 12th day post tumour cells transplantation, a tumour growth is seen in some of the animals in which a significant decrease of the primary humoral immune response is established. In animals without tumour growth immune response is similar to that of the control group. There is an exception for the animals with plasmocytoma in which the immune response is almost blocked, even in the absence of a palpable

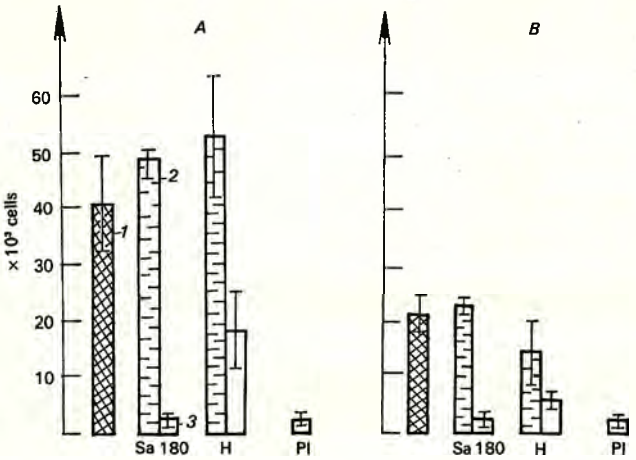


Fig. 1. PFC by sarcoma 180 Crocker, hybridoma and plasmocytoma on the 12th day from the transplantation of tumour cells
A – PFC per spleen; *B* – PFC per 10^8 spleen cells
1 – control; *2* – without tumour growth; *3* – with tumour growth

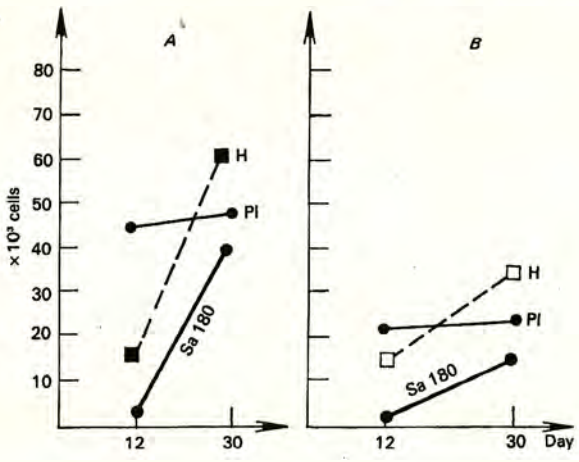
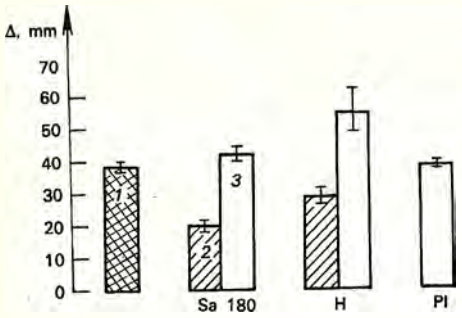


Fig. 2. Distribution of PFC by sarcoma 180 Crocker, hybridoma and plasmocytoma on the 12th and 30th day from transplantation of tumour cells
A – PFC per spleen; *B* – PFC per 10^8 spleen cells



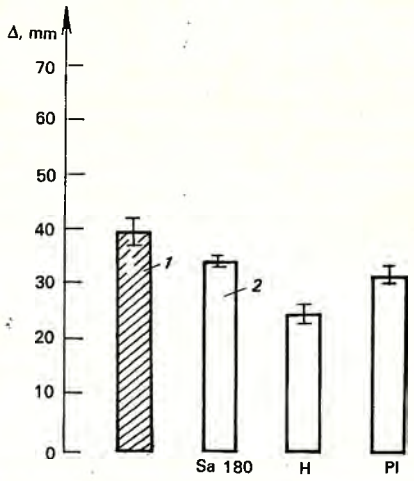


Fig. 3. Delayed type hypersensitivity by sarcoma 180 Crocker, hybridoma and plasmocytoma on the 12th day from transplantation of tumour cells
1 – control (DNFB); *2* – with tumour growth; *3* – without tumour growth

Fig. 4. Delayed type hypersensitivity by sarcoma 180 Crocker, hybridoma and plasmocytoma on the 30th day from transplantation tumour cells
1 – control (DNFB); *2* – without tumour growth

tumour growth. Immune response effect from the tumour growth is checked on the 30th day – after an immunization with SRBC on the 25th of the experiment. Results have been similar to these of the 12th day for sarcoma 180. In animals with hybridoma the number of antibody-forming cells is significantly higher than in the control group. While for those with plasmocytoma, the number of antibody-forming cells does not differ from the control group. Comparing the results of the two periods – 12th and 30th day after tumour cells inoculation we found out that the number of antibody-forming cells increases on the 30th day for the three types of tumours and especially for the hybridoma (Fig. 2).

The dependence of the immune response from tumour growth is also expressed by the reaction of delayed type hypersensitivity. This reaction on the 12th day after inoculation of tumour cells is significantly decreased in animals with well developed sarcoma 180, while in animals without a palpable tumour it does not vary from that of the control group. In all animals with plasmocytoma tumour growth is not registered on the 12th day of tumour cells inoculation, which shows a preserved cellular immune response (Figs 3 and 4).

Discussion and conclusion

There are some difficulties exist in the evaluation of the immunological adaptation to the neoplastic process. On the one hand, the immune system recognizes the tumour cells as alien, while, on the other hand, these tumour cells overcome the immune control and begin to grow progressively. In this way tumour growth suppresses the immune response [13].

In this research the inhibitory effect of sarcoma 180 on the immune response is obvious. This tumour inhibits the immune response from the beginning to the end of tumour development. Sarcoma 180 Crocker is described by Манолов [9], as a tumour that has undergone isogenic evolution and therefore represents a nonspecific tumour "stem line" applicable to different species of mice. Due to this evolution the tumour overcome the immune control of the body and gives a neoplastic growth, suppressing the immune responses.

The analysis of the results for plasmocytoma Sp-2/0 Ag 14 shows that on the 12th day the humoral immune response is significantly decreased even though there is no tumour growth. Two days after this period the first palpable tumour nodes appears. The tumour cells during this period are in a "dormant state" and the suppression of immunity is the probably sign for the beginning of the tumour growth [6].

Jorgensen, Hannestad [4] accept that the secretion of myeloma proteins in plasmocytoma TPC causes idiotype specific immune response of the T-suppressor cells which inhibits the production of antibodies. On the other hand, according to Boyd, Schrader [1] myeloma proteins can inactivate the B-cells by direct intercalation, which leads to functional inactivation of antibody-forming cells in the host and a specific immune tolerance.

In animals with hybridoma tumour on the 12th day a reduction of the number of the PFC in the spleen is observed. This shows that the allogenity of the tumour does not play a significant role. Most probably of greater importance here is the presence of components of normal singeneic cells which are tumour carriers in mice. There are sufficient data in the literature showing, that an alloantigene conjugated with autologica cells (SRBC etc.) experiences a tolerant action on the immune system [11]. In our case the tumour associated-antigens of plasmocytoma Sp-2/0 Ag 14 act as alloantigens and this explains the presence of an inhibitory effect on PFC.

During this period, however, the delayed type hypersensitivity infiltrative reaction is well expressed. It is well known that this reaction gives a general evaluation of the immune status giving information about the level of the T cell-mediated immune response. The result observed on this case is an index of a still active cell mediated immunity.

The data for the duration of the inhibitory effect on immune response are of great interest. On the 30th day the inhibitory effect is still present for sarcoma, while for animals with hybridoma tumour there is an augmentation of the immune response to SRBC. This is difficult to explain. It resembles the immune suppression and the restoration of the immune response during pregnancy, when the semiallogeneic foetus reaches the full development of immune response [10]. For animals bearing well developed plasmocytoma the number of PFC is higher, than that on the 12th day. Терещенко, Сараева [12] explain the increase of PFC of fully developed tumours with the biological specifity of the different tumour models. In connection with antibody formation different results are announced in the literature. For example, Жеромский, Гурны [8], Балбук [7] the well developed tumours cause an inactivation of T-lymphocytes, by increasing the level of B-cells.

Of the results of the 2 examination periods systematized it will be established that the sarcoma growth render a well expressed inhibitory effect upon the both immune responses on the 30th day post transplantation, while the hybridoma and plasmocytoma affect only the cell-mediated immune response. As it is well known,

however, the cell-mediated response is with a fundamental significance in the antitumor immunity, while the humoral immunity has a contradictory character. In conclusion, the effect of tumour growth on the immune response depends of the type of the tumour and the stage of its development.

References

1. Boyd, A., J. Schrader. Mechanism of effector cells blockade. – Cell Immun., 73, 1982, 179-191.
2. Bratanov, M., S. Dimitrova, A. Neronov, I. Goranov. Experimental induction contact dermatitis – morphometricity and micrometricity investigation. – Comp. Rend. Acad. Bulg. Sci., 36, 1981, 385-389.
3. Dresser, D., G. Greaves. Assays for antibody producing cell. – Cell Immunol., 2, 1973, Ch. 72.
4. Jorgensen, T., K. Hannestad. Specificity of T- and B-lymphocytes for myeloma proteins. – Eur. J. Immunol., 315, 1977, 426-431.
5. Oettgen, H. Tumour immunology. – Behring Inst. Mitt., 63, 1979, 80-112.
6. Агеенко, А., С. Гордиенко, О. Саканделидзе. Иммунный ответ при опухолевом росте. – В: Иммунитет и терапия экспериментальных опухолей. Кишинев, Штиница, 1982.
7. Балбук, Г. Иммунологическая реактивность и противоопухолевая резистентность организма. – В: Иммунотерапия опухолевых заболеваний. Кишинев, Штиница, 1983, 8–25.
8. Жеромский, Я., М. Гурны. Результаты и значение иммунологического мониторинга для оценки прогноза и эффективности лечения больных раком легкого. – В: Иммунология опухолей. Рига, Зинатне, 1982, 130–138.
9. Манолов, Г. Генетични и биологични проучвания върху трансплантационни карциноми на млечна жлеза и сарком 180 у мишки. Канд. дис., С., НИО – МА, 1967.
10. Петров, Р. Иммунная система и рак. – В: Иммунология и иммуногенетика, М., Медицина, 1976.
11. Петров, Р., И. Дозморов, А. Левин, Г. Луценко, И. Николаева, Л. Сеславина. Стимулирующее влияние малых количеств лимфоцитов на аллогенные стволовые клетки. – Иммунология, 2, 1983, 38–42.
12. Терещенко, И., З. Сараева. Феномен бляшкообразования в присутствии опухолевого антигена. – Вопр. онкол., 2, 1979, 66–67.
13. Фукс, Б. Мембранотоксическая активность опухолевой клетки. – Иммунология, 1, 1982, 87–88.