The NK Function Elucidated with Respect to Effector Cells, Target Cells and Other Immunological

In vitro Tests

Erik KRISTENSEN*

Fibiger Laboratory

The microcytotoxicity assay and the leukocyte migration inhibition test were unable to reveal any specific reactivity in patients suffering from localized skin melanoma. The non-specific reactivity measured by the microcytotoxicity assay, the so-called NK activity was found to be associated to the cells bearing Fc receptors, irrespective of T and non-T identity. Further more the NK activity depends on the target cell used (established cell lines versus short-term cultures) and the lymphocyte/target cell ratio. The NK activity measured by microcytotoxicity assay and the leucocyte migration inhibition test were compared by simultaneous implementation of the two test systems. No correlation between significant leucocyte migration inhibition and NK activity was found. In a tumor neutralization test work out in a nude mouse model, it was found that patient lymphocytes decreased the number of tumor takes and increase the latency period. However, the specificity of these reactions were uncertain.

(Key Words: NK activity, Fc receptors, Target cells, Leucocyte migration inhibition test, Tumor neutralization test)

INTRODUCTION

In animal experiments it is well documented that chemically and virally induced tumors evoke a specific immune response in the tumor-bearing host. In contrast the data on tumor-immune reactions in human cancer are much less convincing. Primarily the scepticism has arisen concerning the direct cytotoxicity test due to the findings that lymphocytes derived from healthy donors exert a cytotoxicity of the same magnitude as that found in lymphocytes from patients suffering from tumors of the relevant type (3, 8, 16, 17). Also results obtained by other in vitro tests have shown lack of specificity (11, 19).

In recent years, however, the non-specific reactivity measured by the direct cytotoxicity test has been in focus for the tumorimmunological investigations. This type of reactivity has been designated natural killing (NK), natural cytotoxicity or spontaneous cell-mediated cytotoxicity (10, 14). NK was originally interpreted as an in vitro phenomenon without certain biological significance, later on experiments have indicated that this, apparently, non-specific cytotoxicity could have a biological function.

The aim of the present article is to summarize the results obtained by different in vitro techniques in a melanoma system with special regard to NK-activity.

*Present address: Erik KRISTENSEN, Medical-gastroenterological Department C, Herlev University, Herlev Ringvej, DK-2730 Herlev, Demark.
MATERIAL AND METHODS

**Melanoma patients.** The lymphocytes, leucocytes and serum samples were obtained from patients suffering from localized skin melanoma. Stage I: localized primary tumor; Stage II: primary tumor and/or regional lymphnode metastases with or without cutaneous satellite metastases.

**Control persons.** For control purpose blood was drawn from healthy donors or patients without malignant diseases.

**Microcytotoxicity assay (MCA).**

The method of Takasugi and Klein (1970) was used as previously described (8, 12).

**Target cells:** Established lines as well as short-term culture have been used. Tumor types: melanoma, colonic carcinoma, bladder carcinoma, sarcoma. Benign cells were derived from skin fibroblasts.

**Effector cells:** Lymphocyte separation was performed by the gradient method after Boyum. Lymphocytes obtaines from single individuals were fractionated by E-rosettes and EA-rosettes.

**Leucocyte migration inhibition test (LMIT).**

This test was performed in the capillary tube technique. Heparinized blood was sedimented by a dextran solution. As antigen source was used live intact cells, which were placed in the culture chamber. Previously, studies of different antigen preparations revealed no differences between 3M KCl extract, fixed intact cells and live intact cells with respect to revealed specificity (11).

**Tumor neutralization test (TNT).**

Some human established tumor cell lines are tumor producing in nude mouse. This phenomenon has been used to compose a tumor neutralization test. In this assay a mixture of tumor cells and human lymphocytes (patient or control), lymphocyte/target cell ratio 10/1, was inoculated subcutaneously on a nude mouse. Tumor growth rate, latency period and tumor takes were registered (7).

RESULTS

In the microcytotoxicity assay it was found that the degree of reactivity measured by lymphocytes obtained from patients suffering from localized melanoma and control persons was similar. This pattern of reactivity was independent of the chosen target cell type.

However, the magnitude of cytotoxic reactivity mentioned above depends on the lymphocyte/target cells (L/T) ratio, i.e. an increasing L/T ratio caused an increase in the cell killing. In MCA the established cell lines exhibit a more pronounced sensitivity to the lymphocytes compared to short-term cultures. This finding is independent of tumor type (8). This type of non-tumor related cytotoxicity is termed NK-activity. Attempts were performed in order to characterize the effector cells responsible for the NK-activity. In the following experiments different lymphocyte subpopulations are used as
effector cells in MCA. From these experiments it appears that Fc receptor-bearing T-cells as well as non-T-cells exerted the most pronounced cytotoxicity. It was also found that free Fc-receptors were a prerequisite for the NK-activity because in blocking experiment, occupation of Fc receptors by IgG-containing immune complexes, isolated Fc-portions or Fc-split products obtained by enzymatic degradation, reduced the cytotoxic levels. However, the reactivity was not abolished, which indicated that other lymphocyte fractions might be involved (9).

In the LMIT it was not possible to demonstrate a specific reactivity. However, there was registered a reactivity, but this was found in both the melanoma and the control group. Furthermore, this reactivity was most pronounced for two control antigens. In simultaneous and comparable experiments the results obtained by MCA and LMIT were compared and there was no relationship between the non-specific reactivity obtained by these two methods (11).

In the TNT it was possible to demonstrate that lymphocytes derived from melanoma patients caused an increase in the latency period and a decrease in tumor takes whereas the rate of tumor growth was uninfluenced. This was only compared with lymphocytes from healthy donors. The experiment was not performed with other cell lines than melanoma, and therefore the specificity of the measured reactivities is not ruled out (7).

DISCUSSION

The scepticism with respect to the specificity of in vitro methods within the field of human tumorimmunology is supported by the present results.

In human tumor immunology a crucial point in test systems is the real value of the target/antigen sources. The main question is: Does there exist tumor-associate antigens (TAA) in human tumors, comparable with the animal model, which will be recognized as foreign structures and evoke an immune response in the patient suffering from a malignant disease. Target/antigen might be obtained from established cell lines, short-term cultures or primary cultures. It is to be expected that primary cultures are most related to the in vivo conditions. However, the employment of these cells might be accompanied by some drawbacks, for example contamination of stromal cells, infiltrating immune cells and immuno-globulin content (6, 18). Cultivated cells might apparently represent a more homogeneous cell population. However, it cannot be excluded that the theoretical TAA are changed during the period of cultivation (4, 5, 20).

Furthermore it is demonstrated that also established cell lines consist of different cell subpopulations and therefore a real homogeneous tissue culture requires cell-cloning (15). Also the cell density in tissue culture has influence on the expression of TAA (1, 2). Finally it should be mentioned that reactivity obtained by different test systems (19) showed a pronounced variability from cell line to cell line and from primary tumor to primary tumor. The explanation of this variability is unknown. It is obvious that such ill-defined target/antigen might imply tremendous difficulties in the comparison of results obtained by different target/antigen preparations. It has been demonstrated that the NK-activity is reduced in patients suffering from malig-
nant skin melanoma. Whether this finding is primary or secondary to the malignancy is still controversial. From an animal model using the mouse mutant beige mouse, which is characterized by impaired NK-activity, it seems that the NK-activity plays an active role in the tumor homeostasis. These findings are very important from a clinical point of view because it has been demonstrated that interferon enhances the NK-activity. This phenomenon is showed most clearly by Langvad and Hyden (13), in an elegant experiment using an extracorporeal perfusion chamber containing insolubilized interferon bound to the chamber walls. In this experiment it has been possible, in a clear cut way, to separate the effect on lymphocytes of interferon from the influence on target cells.

A hypothesis could be, that the high NK activity demonstrated in MCA using established cell lines as target, is caused by a high interferon production of the effector cells. This production is induced by the established cell lines whereas short-term cultures are unable to induce such a interferon production. The same explanation could explain the findings of Kristensen (10), who found that cell lines, which were non-tumorogenic in nude mice, were also sensitive for NK activity in vitro.

In conclusion whether there exist a real tumourspecific immuneresponse in human cancer patients is still under debate. It might be possible that human TAA are to weak to evoke the immune system. The relationship between a tumourspecific reactivity and the apparently non-specific NK activity is unknown.

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