Urological Research 6, 207-209 (1978)

Urological Research

Cellular Cytotoxicity in Transitional Cell Carcinoma of the Human Urinary Bladder – A Summary

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Summary. The cytotoxicity in vitro of peripheral blood lymphocytes from patients with carcinoma of the urinary bladder (TCC-bladder) against allogeneic target cells from established cell lines was studied by the ^{51}Cr-release assay. Lymphocytes from both untreated and treated TCC-bladder patients have a significantly elevated mean cytotoxicity to TCC-bladder target cells. Tumour cell destruction by lymphocytes from TCC-bladder patients shows a clear disease related specificity. In TCC-bladder patients a superimposed cytotoxicity exists, probably reflecting reactions against one or several tumour - associated antigens. In treated patients this cytotoxicity may be masked by higher incidence of cross reaction.

Key words: Bladder carcinoma - Cell mediated immunity - Lymphocyte cytotoxicity - Tumour associated antigens.

Tumours in experimental animal systems are frequently immunogenic and may elicit an immune response in the tumour bearing host. This immune response has been shown to affect tumour growth in several different ways. For spontaneously arising malignancies in man this situation is not at all clear (7). Although available evidence suggests that patients do mount immune responses against their tumours, the specificity and significance of these reactions is controversial (2). Therefore, in human tumour immunology the major task at present is to elucidate the specificity of the patients' immune response against their tumours. Clarification of this problem is also thought to provide a basis for using the patients' own response as possible diagnostic or prognostic means for monitoring the disease. A second major task in this area is to establish the possible protective role of different immunological effector mechanisms during different phases of disease.

In transitional cell carcinoma of the urinary bladder (TCC-bladder) we have attempted to elucidate these problems by investigating the cell mediated destruction of bladder carcinoma cells in vitro, using the patients' blood lymphocytes as effector cells. This reaction is considered to reflect an important immune mechanism responsible for the initiation of tissue damage occurring in vivo. This paper summarises the results of investigations performed in our laboratory. For details and references the original articles should be consulted.

CYTOTOXICITY ASSAYS

In the initial studies, lymphocyte mediated cytotoxicity was studied by means of the microcytotoxicity assay in which lymphocytes and tumour cells are incubated together for 24-48 h and the number of surviving tumour cells is then assessed by counting (11). Studies of a large quantity of material indicated a) that TCC-bladder target cells were damaged more extensively by bladder cancer patients' lymphocytes than by those from clinical controls or healthy donors, and b) that destruction of normal bladder epithelial cells or of unrelated tumour cells by the TCC-bladder patient's lymphocytes was equal to that obtained with the lymphocytes of the controls. In

0300-5623/78/0006/0207/$01.00

essence, these results suggested the occurrence in TCC-bladder patient's of a disease related cytotoxicity displayed by the patient's lymphocytes (3, 4, 5). In addition, later investigations also revealed certain correlations between this in vitro reactivity of the patient's lymphocytes and the clinical course of their disease (8, 9). The most important point emerging from these studies was that positive or negative lymphocyte reactivity in vitro may be of entirely different significance, dependent on phase of disease, clinical conditions, or type of therapy at the time of testing.

During recent years the usefulness of the microcytotoxicity assay for monitoring patients has been the subject of extensive debate (1, 6). In particular, it has been pointed out that this procedure provides only relative measures of both specificity and intensity of a lymphocyte reaction, depending on the baseline used for computing the results. Moreover, the assay requires long incubation periods which in certain instances may lead to either false positive or false negative reactions. It was therefore deemed important to reinvestigate the problem of lymphocyte cytotoxicity in bladder carcinoma by means of an independent assay procedure. For this purpose, an adaption of the ^{51}Cr-release assay was used in which release of the isotopic marker from labelled tumour cells after the addition of lymphocytes provides a measure of tumour cell destruction. This assay procedure measures target cell lysis more directly than the microcytotoxicity assay, requires shorter incubation periods and hence allows for a more accurate assessment of both intensity and specificity of lymphocyte induced tumour cell destruction.

Specificity of the Cytotoxic Reaction

In the studies to be described, 4 groups of lymphocyte donors were studied, each group comprising approximately 20-30 individuals. These were

1) untreated TCC-bladder patients, all with tumours of clinical stages T1 - T3

2) TCC-bladder patients treated with local radiotherapy from 1 - 12 years before testing.

3) age and sex matched clinical controls, mostly patients with carcinoma of the prostate, also untreated for their cancer.

4) healthy donors.

Purified blood lymphocytes from all donors were tested against a small panel of allogeneic target cells from established cell lines, all of about equal susceptibility to cell mediated lysis but of different origins (2 of TCC-bladder origin, 2 from normal bladder epithelium, 1 from colon carcinoma and 1 from malignant melanoma). Lymphocyte mediated lysis of these cells was assessed against the background provided by the lymphocyte free medium controls (12).

Using the ^{51}Cr-release assay, lymphocytes from all four groups were frequently found to lyse target cells of various origins and there was a considerable scatter in the intensity of cytotoxicity exhibited by individual donors. However, when taken as a group, lymphocytes from both untreated and treated TCC-bladder patients had a significantly elevated mean cytotoxicity to TCC-bladder target cells. For the control donors this was not the case. Thus, these studies confirm previous findings that tumour cell destruction by lymphocytes from TCC-bladder patients shows a clear disease related specificity. While this is true for untreated patients having tumours of clinical stage T1-T3, patients with tumours of stage T4 frequently show a suppressed reactivity, similar to what was also found in the earlier studies (3, 8).

Influence of Therapy on the Cytotoxic Reaction

Application of the ^{51}Cr-release test also makes it possible to assess "cytotoxicity profiles" of individual donors; These studies showed that the cytotoxicity of lymphocytes from individual donors in most instances is selective, i.e. it comprises only one or a few target cell types on the test panel. Thus, for untreated patients in the TCC-bladder group, cytotoxicity to TCC-bladder targets was not correlated with that to any of the other target cells, However, for the treated patients who were a very heterogenous group, this was different in that cytotoxicity to TCC-bladder targets was correlated with that to target cells. of normal bladder epithelium origin. Taken together, our results suggest that the cell mediated cytotoxic reactions detected in this way reflect the occurrence in both patients and controls of multifactorial immune responses against a variety of antigens, differently expressed on target cells of different types. In TCC-bladder patients there exists a superimposed cytotoxicity against one or several antigens seemingly not expressed on unrelated tumour cells or normal bladder. This cytotoxicity is most easily revealed in untreated patients whose tumour burden is limited. In patients treated with radiotherapy, this disease related cytotoxicity may be masked by additional reactions against other antigens common for several cell types. Because of this complexi-

ty, the lymphocyte mediated cytotoxicity can not readily be used to monitor individual patients at present. However, methods are now available to further clarify the molecular basis of the disease related reactions and this will be of help for their application as diagnostic or prognostic tools in the clinical laboratory (12).

Nature of the Effector Lymphocytes

Investigation of the nature of the effector lymphocytes recently indicated that several different effector mechanisms are responsible for the lymphocyte mediated cytotoxicity discussed above. While most of the reactions against allogeneic tumour cells seem to be displayed by lymphocytes which need humoral antibodies for being specifically cytotoxic, antibody independent but nevertheless specific cytotoxic T-lymphocytes may sometimes efficiently destroy autochthonous tumour cells. In addition, so called "natural killer" lymphocytes, destroying tumour cells with a low degree of selectivity may also be present in the blood of these patients. Since these different effector mechanisms vary in efficiency and biological significance, it remains to be established how they balance each other in the course of the disease and which of them, if any, has protective significance (10, 13).

Acknowledgements. This work was supported by grant no. 365-B76-08XB from the Swedish Cancer Society.

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