Augmentation of Natural Killer Cell Activity after Arterial Embolization of Renal Carcinomas

August Bakke, Jan H. Göthlin, Svein A. Haukaas, and Terje Kalland

ABSTRACT

Preoperative embolization of the renal artery has been reported to improve the survival of patients with advanced renal carcinomas compared to operative treatment only. To investigate possible immunological consequences of tumor embolization, natural killer (NK) cell activity in peripheral blood was investigated immediately before and at different time intervals after occlusion of the renal artery by insertion of a metal coil. A slight increase in NK activity could be observed 24 hr postembolization while a marked augmentation was seen after 48 hr. The high NK activity persisted up to 96 hr after embolization, the last time period included in the study. Two patients undergoing the same procedure but in whom embolization was unsuccessful showed no alteration in NK activity.

It is suggested that interferon produced by macrophages activated by the necrotizing tumor might be responsible for the augmentation of NK activity.

INTRODUCTION

Patients with primary renal adenocarcinomas with metastases at the initial presentation constitute a therapeutic challenge for the urological surgeon as well as for the medical oncologist. Renal carcinomas have generally been found to be refractory to chemotherapeutic agents, radiation, and hormonal manipulation (10, 29). The minimal benefit derived from nephrectomy in the presence of metastatic disease (11, 12, 13) has prompted the search for alternative procedures, among which has been the embolic occlusion of the renal circulation (1, 2). Following renal artery occlusion, stabilization of metastatic disease and in some cases complete regression of metastases have been observed (21, 30).

Swanson et al. (30) found that embolization followed by nephrectomy and progestin therapy significantly improved the survival of patients with lung metastases, although only historical controls were used in the study.

The mechanisms involved in this improved survival after embolization of renal carcinomas are not known. Immunological factors have been suggested to be of importance based on the assumption that tumor infarction may release high amounts of tumor antigens into the circulation, thereby triggering an antitumor response (4, 6, 30).

Numerous data accumulated during the last few years clearly indicate that nonsensitized lymphoid cells can be effective in the destruction of tumor cells (10). The cells primarily responsible for these effects are termed NK cells. Recent evidence indicates that NK cells may be implicated as effector cells in immunological surveillance of neoplasia and also may be of major importance in the control of metastatic spread of cancer cells (9, 15, 16). Mice depleted of NK cells in various ways have increased incidence of carcinogen-induced tumors and a higher take of tumor cells after transplantation of tumor cell lines (14, 17, 31). Tumors transplanted to nude mice which have a high NK activity rarely metastasize (31), and there is a direct correlation between the level of NK activity in different mouse strains and the short-term survival of isotopically labeled tumor cells using a lung colony assay (25). Moreover, the NK-deficient beige mouse has a marked increase in the metastatic seeding of tumor cells from transplanted tumors. The finding of an increased resistance to NK-mediated lysis of metastatic cells compared to cells from the primary tumor may also point to a role of NK cells in the control of metastases (8).

The present study is dealing with the possible involvement of NK cells in the improved survival after arterial embolization of renal carcinomas.

MATERIALS AND METHODS

Patients. Thirteen patients with a confirmed radiological diagnosis of renal carcinoma by renal angiography were included in this study, ranging from 49 to 83 years of age, with a mean age of 62 years. Staging of the disease was by the modification by Robson et al. of the classification of renal carcinomas of Flocks and Kadesky (27). Three patients were in Stage 1, 2 patients were in Stage 3, and 8 patients were in Stage 4. The patients in Stages 1 and 3 were operated on, and the diagnosis was confirmed histologically. The operations were done 5 to 7 days after the embolization procedure. All immunological evaluations were done before surgery, and the patients received no treatment other than occasional analgesics. The 8 patients in Stage 4 had all known metastases at the embolization time, and they were not operated on. In 2 patients, embolization was unsuccessful. NK activity in peripheral blood was investigated immediately before and at different time intervals after embolization (see "Results").

Embolization Procedure. Subsequent to renal angiography, the main arteries supplying the tumor were embolized by insertion of a metal coil as described by Gianturco et al. (7).

Effector Cells. Mononuclear cells from peripheral blood were separated on Lymphoprep (Nyegaard & Co., Oslo, Norway) according to the method of Bayum (3). The cells were washed 3 times in phosphate-buffered saline (pH 7.4) and resuspended in RPMI 1640 with 20% autologous or pooled human AB serum. In preliminary experiments, plastic-adherent cells were found to exert only negligible cytotoxic activity in the present assay system, and filtration through nylon wool increased the cytotoxic activity. Monocyte-mediated cytotoxicity thus does not seem to contribute in the present assay system, and all experiments in the present paper are performed with lymphoprep-separated effector cells without further purification. The percentage of cytotoxicity was close to linear at effector/target ratios between 100 and 10.

Target Cells. Molt-4, a T-lymphoblast cell line originally established from a patient with acute lymphoblastic leukemia (20), was maintained as stationary suspension culture in RPMI 1640 supplemented with...
10% fetal calf serum and gentamicin (50 µg/ml). The target cells were regularly screened for the presence of Mycoplasma.

Cytotoxicity Assay. Target cells were labeled by incubation of 5 × 10^6 cells in 0.5 ml complete medium with 100 µCi Na_251CrO_4 (specific activity, 200 to 400 mCi/mg; The Radiochemical Center, Amersham, England) for 45 min at 37°. The cells were washed twice in 50 ml phosphate-buffered saline and resuspended in RPMI 1640 to 10^5 cells/ml. One hundred µl target cells were admixed to 100 µl effector cells at different concentrations in Greiner round-bottomed microtiter plates to give effector:target cell ratios of 100:1, 50:1, and 10:1 and incubated at 37° in 5% CO_2 for 5 hr. After centrifugation at 200 × g for 10 min, 100 µl of the supernatant were pipetted off, and radioactivity was determined in a Searle 1185 gamma spectrophotometer. Spontaneous release was determined by incubation of labeled target cells in medium only and varied from 10 to 20% of total radioactivity. Total radioactivity was determined by counting samples of the labeled target cells. Variation between triplicates was below 5%. Percentage of cytotoxicity was determined by:

\[
\frac{\text{Test cpm} - \text{spontaneous cpm}}{\text{Total radioactivity} - \text{spontaneous cpm}} \times 100
\]

The NK activity before and 48 hr after embolization was compared using Student's t test.

RESULTS

In initial studies, the time dependence of NK activation of peripheral blood lymphocytes was investigated in 3 patients undergoing arterial embolization of renal carcinomas. NK activity was studied immediately before embolization as well as at 24-hr intervals up to 96 hr after the procedure (Chart 1).

While a slight increase in NK activity could be observed as early as 24 hr postembolization, a profound augmentation was seen after 48 hr. This high level was retained essentially throughout the time period studied.

In further studies, the level of NK activity at maximum stimulation (48 hr) was compared with the preembolization level; thus, each patient served as his own control. In Chart 2, the effect on NK activity of tumor embolization of 11 consecutive patients with renal carcinomas is shown. All patients except 2 showed increase of NK activity after embolization. Comparing NK activity before and 48 hr after embolization revealed a statistically significant enhancement (p < 0.01). One patient had a sharp drop in activity. This single exception was a patient with an unusual preembolization NK activity from whom an open biopsy of a bone metastasis had been taken 2 days earlier. Not included in Chart 2 are 2 patients in whom embolization was unsuccessful and who showed no alteration in NK activity (15.7 and 25.1% cytotoxicity before and 11.9 and 26.0% cytotoxicity 48 hr after manipulation, respectively). The NK activity was assayed both in autologous serum and in pooled AB-positive serum. There was no difference in activity with the 2 different serum sources, either before or 48 hr after embolization (Table 1).

However, 24 hr after embolization, lymphocytes incubated with the patients’ own serum showed a somewhat higher activity than did those assayed in the presence of AB serum, although this effect was not statistically significant.

DISCUSSION

The present study demonstrates that therapeutic arterial embolization of renal carcinomas is accompanied by a significant increase in the NK activity of peripheral blood lymphocytes. While it is not possible to include a normal patient population as control in this study, the lack of increase in NK activity of 2 patients where embolization was unsuccessful indicates the embolization-related nature of the observed NK activation. Moreover, pre- and postembolization assay of dif-

![Chart 1](chart1.png)

**Chart 1.** Time dependence of NK activation after arterial embolization of renal carcinomas in 3 patients (C, D, O). Effector:target ratio, 50:1; AB serum.

![Chart 2](chart2.png)

**Chart 2.** NK activity in peripheral blood of patients immediately before and 48 hr after arterial embolization of renal carcinomas. Horizontal bars, means. Effector:target ratio, 50:1; AB serum.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>No. of patients</th>
<th>% of cytotoxicity a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Autologous serum</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>16.9 ± 6.1</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>28.0 ± 5.5</td>
</tr>
<tr>
<td>48</td>
<td>11</td>
<td>38.6 ± 6.8</td>
</tr>
</tbody>
</table>

a Effector:target cell ratio, 50:1.
ferent patients were run simultaneously, which would tend to prevent day-to-day variation in the assay to influence the results significantly. Although some day-to-day variation during assay of NK activity is commonly recognized, the experience from both this laboratory and others (22) is that NK activity in the individual patient is remarkably stable. It should be emphasized that the possible role of NK cells in an antitumor response is limited by their capacity to deal only with a small number of tumor cells (28). Thus, their major importance in tumor-bearing patients is not toward the primary tumor but rather against freshly established micrometastases and tumor cells traveling through the peripheral blood compartment during the metastatic process. The augmented NK activity induced by preoperative embolization of renal carcinomas may also be of importance in the arrest of tumor cells released during resection of the primary tumor. The mechanisms of the increased embolization-induced NK activity are not known and may be related to necrosis per se or more specific to the renal carcinoma. Carmignani et al. (4) found no alterations in the total number of T- and B-lymphocytes after embolization of renal carcinomas. Whether the augmented NK cell activity in peripheral blood is the result of a general increase in the number or lytic capacity of NK cells or merely a redistribution of effector cells to peripheral blood is not known. Homing effects on NK cells have been described for, e.g., corticosteroids which lead to a reduction of NK cells in peripheral blood in the human (24). Interferon is well recognized as a major regulator of NK activity in vivo, and most agents that have been reported to increase NK activity exert their effects via interferon, although some exceptions may exist (18, 19). Macrophages have been shown to produce interferon in response to stimulation with poly(inosine-cytidylic) and have also been reported to produce interferon when challenged with tumor cells (5, 23). The major host cells surrounding the necrotic areas after embolization of renal carcinomas are macrophages. These cells may not only act as scavenger cells engulfing necrotic material but may also be activated to produce, e.g., interferon, which in turn may be responsible for the increased NK activity accompanying embolization of renal carcinomas. However, it should not be excluded that the necrotizing tumor may release soluble factors able to enhance NK activity directly.

In favor of a serum factor being the mediator of the increase in NK activity is the observation that effector cells incubated in the presence of autologous serum 24 hr after embolization show a tendency to a more vigorous response than do effector cells incubated in AB serum. This difference is abolished when maximal stimulation of NK activity is reached. The kinetics of activation is also consistent with this hypothesis. Serum batches from the patients presented in this study are presently analyzed for the presence of interferon as well as for other potential NK-activating serum factors. Most patients develop fever as part of a postinfarction syndrome. Hyperthermia has also been reported to improve after embolization as judged by the cutaneous response to recall antigens and the proliferative response of peripheral blood lymphocytes to T-mitogens (30). No thorough studies on the effect of hyperthermia on human NK activity have to our knowledge been published.

While several questions regarding the nature of the embolization-induced augmentation of NK activity remain, this phenomenon deserves further attention as a possible mediator of the increased survival and casuistic reports of regression of metastasis observed after preoperative embolization of renal carcinomas.

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