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Enhancing Tumor Implantation and Growth Rate of Ramos B-Cell Lymphoma in Nude Mice

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The ability of a human B-cell lymphoma cell line to grow subcutaneously as tumors in nude mice was investigated. The effect of pretreating mice with cyclophosphamide or whole-body irradiation (WBI) was compared with no pretreatment of the mice. Both methods of pretreatment resulted in a higher tumor implantation rate, compared with that for non-pretreated controls. In mice that underwent WBI-pretreatment, a tumor implantation rate of 100% was observed, whereas mice pretreated with cyclophosphamide had a tumor implantation rate of 80%. In non-pretreated control mice, an implantation rate of only 50% was observed. Three weeks after injection, tumor size was significantly larger in mice of the pretreated groups, compared with that in mice of the group that did not receive pretreatment. Furthermore, particularly in the group pretreated with WBI, the tumors grew more synchronously, compared with tumors in the control group. Results of this study indicate that pretreatment with cyclophosphamide or WBI improves the tumor implantation rate of Ramos cells in nude mice, providing a workable animal model for studying human B-cell lymphoma.

Immunotherapy with monoclonal antibodies (mAbs) and radioimmunotherapy with radiolabeled mAbs are new treatment modalities for patients with recurrent or widespread malignancies. Patients with malignant B-cell lymphomas may respond especially well to this kind of treatment (1). Mice with human tumors are used as models to optimize this kind of treatment (e.g., to determine which monoclonal antibody and which radionuclide would be the best choice for this kind of treatment). Malignant lymphomas are usually disseminated in vivo, as represented by mouse models using severe combined immune deficient (SCID) mice (2-5). For evaluation of radioimmunotherapy, only one circumscript, subcutaneous tumor lesion that allows evaluation of tumor growth, is preferable. For this purpose, athymic BALB/c mice bearing a subcutaneous lymphoma xenograft are used in most animal studies (6-12). Transplantation of human lymphoma cells in nude mice is difficult (6). Vuist and co-workers reported failure of several Burkitt's lymphoma cell lines (BJAB, EB3, Ramos, Jiyoye, and Namalwa) to grow subcutaneously. Only the Daudi cells eventually grew in BALB/c nude mice younger than eight weeks (6). Leonard and co-workers observed that, after subcutaneous injection of Namalwa cells, none of the inoculated BALB/c nude mice developed a tumor (7). In nude mice, numbers of B cells, natural killer (NK) cells, and macrophages are at least similar to those in immunocompetent mice (13). This residual immunologic defense is considered to prevent growth of some human tumors in nude mice (13-15).

To enhance induction and growth rates of tumor xenografts in nude mice, the following immunosuppressive strategies can be used: pretreatment with cyclophosphamide (13, 14), and pretreatment with whole-body irradiation (WBI) (7, 13, 15). In the study reported here, the effect of both methods on tumor implantation and growth rate of Ramos cells in nude mice was investigated.

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Materials and Methods

Animals and husbandry. Female, home-bred mice of the seventh generation BALB/c-nu inbred strain were studied. The mice were serologically screened for known rodent viruses according to recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) and were found negative for these viruses. The mice were housed behind strict barriers to maintain the specific-pathogen-free status. At the start of the experiment, the mice were six to eight weeks old and weighed 20.6 ± 1.0 g. The mice were housed in wire- and filter-topped Macrolon type-II cages (375 cm^2, Techniplast, Utrecht, The Netherlands), five mice per cage, with autoclaved sawdust bedding (Woodyclean $^3/_4$, BMI, Helmond, The Netherlands). Cages were cleaned once a week. Food pellets (RMH-GS, Hope Farms, Woerden, The Netherlands) and acidified tap water (HCl, pH 2.5 to 3) were provided ad libitum. The animal room had a controlled photoperiod (7 a.m. to 7 p.m., lights on; 7 p.m. to 7 a.m., lights out), temperature (21°C), relative humidity (± 55%), and ventilation (15 air changes/h).

Animals were treated and used humanely, according to the requirements of Dutch legislation. This experiment was approved by the internal Review Board for the use of laboratory animals.

Cell culture. Ramos cells (CRL-1596, American Type Culture Collection, Manassas, Va.) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and 100 m*M* L-glutamine. Cell cultures were kept in logarithmic growth phase and were maintained in a humidified 5% CO_2 atmosphere at 37°C. On the day of tumor cell inoculation, cell suspensions were centrifuged in 50-ml tubes at 500 ×*g* during 5 min, washed once in RPMI 1640 medium, and resuspended in RPMI 1640 medium (10^8 cells/ml), then were injected into mice within 1 h.

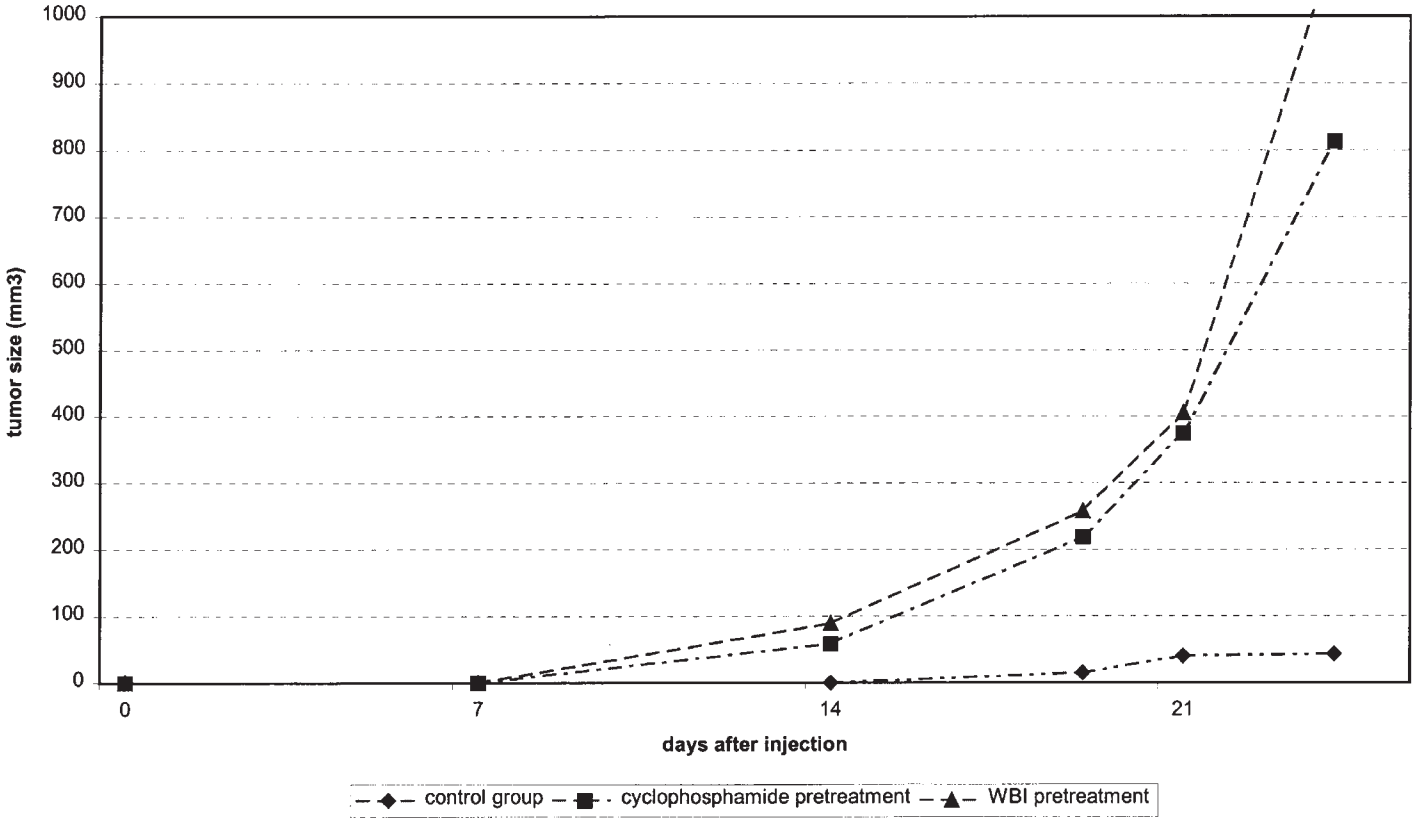

Figure 1. Mean tumor sizes in the three groups of mice.

Experimental procedure. Mice were randomly divided into three groups of 10 mice/group. One group served as a control group and did not receive any pretreatment. The second group received cyclophosphamide (100 mg/kg of body weight) intraperitoneally (14). Cyclophosphamide (Endoxan-Asta, Dagra Pharma B.V., Diemen, The Netherlands) was obtained from the Department of Clinical Pharmacy. The third group received WBI by use of external beam radiation with 15 MeV electrons at a dose of 6 Gy, using a linear accelerator (Saturne 42F, GE Medical Systems, Buc, France) (2,15). During the WBI procedure, mice were kept in plexiglass boxes. Twenty-four hours after pretreatment, 10^7 Ramos tumor cells in 0.1 ml were injected subcutaneously into the right flank of the mice.

Tumor growth was monitored by use of caliper measurements in three dimensions, and was performed by the same investigator (JM). Tumor growth was classified as negative, positive (but not yet measurable), or measurable. As soon as tumors were measurable, measurements were carried out three times a week. Tumor volume of measurable tumors was calculated by assuming that tumors were ellipsoids (16), and the mathematical formula for calculating the volume of an ellipsoid ($^1/_6$ π × length × width × height) was used. Minimal detectable tumor volume was 50 mm^3. Mice were considered suitable for further experiments when tumor volume was between 50 and 1,000 mm^3.

Analysis of data. Tumor volumes measured three weeks after inoculation were compared statistically using Wilcoxon tests.

Results

Figure 1 depicts mean tumor size in each group during three weeks after inoculation with tumor cells. Three weeks after injection, tumor sizes were significantly larger in both groups, compared with that for the control group ($P = 0.01$ for pretreatment with cyclophosphamide, $P < 0.01$ for pretreatment with WBI). There were no significant differences between the group pretreated with cyclophosphamide and the WBI pretreated group. Tumor behavior and tumor implantation rate markedly varied among the three groups. Therefore, the three groups are discussed separately.

Control group. In the control group, tumor growth was observed macroscopically 19 days after tumor induction in three mice (mean ± SD, 50 ± 10 mm^3). Tumor growth in these mice was relatively slow. On day 31 after tumor cell inoculation, all mice had a tumor with mean volume of 310 ± 322 mm^3. Mean time from inoculation to first appearance of a tumor was 22 days. In five of these mice, tumors regressed. Thus, the tumor implantation rate in this group was 50%.

Pretreatment with cyclophosphamide. Macroscopic tumor growth in the mice that received pretreatment with cyclophosphamide, started 14 days after tumor cell inoculation, when four tumors appeared. On day 19 after tumor cell inoculation, all mice had a tumor, with mean volume of 213 ± 230 mm^3. Two tumors regressed; thus, the tumor implantation rate in this group was 80%.

Pretreatment with WBI. In the group of mice that were irradiated prior to tumor inoculation, tumor growth became apparent 14 days after inoculation in nine mice, with mean volume of 100 ± 111 mm^3. On day 19, all mice had a tumor, with mean volume of 257 ± 132 mm^3. Regression of tumors was not observed; thus, the tumor implantation rate in this group was 100%.

Discussion

Tumor growth profiles of the groups indicate that both pretreatment methods enhanced tumor survival and growth rate. Tumor survival rate increased from 50% to 80% in association with cyclophosphamide, and to 100% in association with WBI. To allow evaluation of various therapeutic strategies, it is essential that tumors do not spontaneously regress. In addition, in both pretreated groups, therapeutic experiments could be started within three weeks after tumor induction. Considering that tumors measuring between 50 and 1,000 mm^3 could be used for further experiments, 50% of the mice pretreated with cyclophosphamide were suitable for actual experiments between days 18 and 21 after tumor cell inoculation. For mice of the WBI-pretreated group, 100% were suitable for further experiments between days 14 and 21. In a subsequent experiment in 20 mice, each pretreated with cyclophosphamide, 80% of the mice (16/20) had developed tumors with suitable size 14 days after inoculation.

In the study reported here, we used athymic BALB/c mice. In most preclinical studies with (radiolabeled) mAbs, athymic BALB/c mice were used (6-12). Athymic BALB/c mice lack functional T cells. The SCID mice have even less residual immunity than do athymic mice; the former lack functional T and B cells. However, both mouse strains still have some NK- cell activity. Since SCID mice are even more immunodeficient than are BALB/c mice, these mice are also used in models of disseminated hematologic malignancies (2-5). In those experiments, the lymphoma cells are injected intravenously, not leading to uniformly comparable tumors. Induction of subcutaneous, human Hodgkin lymphoma xenografts in SCID and athymic mice were described by Kapp and co-workers (17). After subcutaneous injection of three Hodgkin lymphoma cell lines in three groups of five athymic mice, tumor growth was not seen in athymic mice. In three groups of five SCID mice, tumors were seen in three of five, three of five, and one of five of these mice. A fourth Hodgkin cell line induced subcutaneous tumors in three of five nude mice and five of five SCID mice. Finally, a fifth cell line did not grow at all in nude and SCID mice (17).

Results of this study indicated that both methods of pretreatment can improve the implantation rate of Ramos tumors in athymic BALB/c mice. Use of WBI-pretreatment was associated with tumor implantation rate up to 100%, rapid induction of tumor growth, and synchronous growth of the tumors. Pretreatment with cyclophosphamide yielded less favorable results, but is easy to carry out, with good tumor implantation rate, 80%, in this experiment.

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