Expression of peanut agglutinin receptors on virus-induced preleukemic cells in mice
(radiation leukemia virus/thymocytes)

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Communicated by Philip Levine, December 19, 1979

ABSTRACT Preleukemic bone marrow and spleen cells of irradiated C57BL/6 mice that were inoculated with the radiation-induced leukemia virus variant D-RadLV differ from autonomous end-stage leukemia cells in the expression of the receptor for peanut agglutinin. As a result, the preleukemic cells are agglutinated by peanut agglutinin and the end-stage cells are not. This observation provides further evidence that preleukemic cells possess surface markers similar to those of the prothymocyte. In vitro and in vivo thymocyte–virus interactions reveal that cells susceptible to D-RadLV transformation are present among the peanut agglutinin-receptor-bearing thymocyte population which has previously been demonstrated to be immunologically immature.

A high proportion of irradiated C57BL/6 mice inoculated with the radiation leukemia virus variant D-RadLV develop T-cell leukemias (1). These leukemic cells have the phenotype of the minor thymus subpopulation, expressing high levels of H-2 alloantigen and relatively low levels of Thy-1 (2). In contrast, preleukemic cells that have the potential to develop into leukemic cells after transplantation into the appropriate recipient mice are present in the bone marrow shortly after intrathymic D-RadLV inoculation (3) and have the characteristics of prothymocytes, lacking the Thy-1 cell surface differentiation antigen (4). Besides this difference in acquisition of the antigen, preleukemic cells also have been found to differ from leukemic cells in their immunizing capacity and in the specific environmental requirements needed for their further proliferation into T-cell leukemias (4, 5).

Here we demonstrate that preleukemic cells differ from leukemic cells by the expression of an additional surface marker, the receptor for peanut agglutinin (PNA), as a result of which the former cells are agglutinated by PNA (PNA+) and the latter are not (PNA−). We also demonstrate that the cells susceptible to D-RadLV transformation in the thymus are among the PNA+ cells.

MATERIALS AND METHODS

Animals. C57BL/6 parental mice (6–8 weeks old) and (BALB/c × C57BL/6)F1 hybrid mice (8–10 weeks old) were used throughout. All mice were raised at the Weizmann Institute Animal Center.

Cell Suspensions. Bone marrow was obtained from the femurs and suspended in phosphate-buffered saline (P/NaCl). Spleen suspensions and thymocyte suspensions were prepared by straining the tissue, in P/NaCl, through nylon gauze. The cells were then washed twice in P/NaCl and resuspended in the same buffer at the required final concentration. Nucleated cells were counted in Tryck’s solution.

PNA. PNA was purified by affinity chromatography on a column of Sepharose-N-[(ε-aminoacaproyl)-β-D-galactopyranosylamine (6).

Cell Separation. Thymocytes were fractionated into two subpopulations as described by Reisner et al. (7). Separation of bone marrow and spleen cells was carried out with the aid of PNA as described by Reisner et al. (8).

Agglutination Test. Agglutination rate was measured at 24°C according to the method of Danon et al. (9) with a Flapiligraph (model D-2, Elmedix Ltd., Tel Aviv, Israel) equipped with a linearization unit. This measurement is based on the fact that transmission of light through a cell suspension is related to the size of the aggregates that are formed upon the interaction of the cells with the lectin.

Transplantation Bioassay. Leukemogenic potential of cell suspensions was tested by the transplantation bioassay method of Haran-Gera (10).

Leukemia Induction. Preleukemic cells were induced by injecting D-RadLV into the thymus of C57BL/6 mice 6–8 weeks old. The virus preparation and technical procedures are described elsewhere (1). End-stage leukemia cells were obtained from thymuses (thymomas) 140–400 days after virus inoculation.

RESULTS AND DISCUSSION

It has recently been shown that PNA, a lectin specific for D-galactosyl-β(1→3)-N-acetyl-D-galactosamine, binds specifically to immature mouse thymocytes (7) as well as to hemopoietic stem cells present among murine bone marrow and spleen cells (8). Mature lymphocytes do not bind the lectin because the receptor is masked by sialic acid. These findings suggested that preleukemic cells having the characteristics of committed precursor T cells may also possess the PNA receptors on their surfaces, whereas on leukemic cells bearing the phenotype characteristics of mature T cells the receptor may be masked by sialic acid and thus be refractory to PNA-induced agglutination (PNA−).

We have compared the agglutination by PNA of D-RadLV-induced leukemic thymocytes (1) (2 × 10⁶ cells in 2 ml of P/NaCl plus 1.5 mg of PNA per 0.4 ml of P/NaCl) to that of thymocytes and cortisone-resistant thymocytes of normal mice under the same conditions. The total normal unseparated thymocyte population, consisting mainly of immature T cells, was strongly agglutinated, whereas the leukemic thymocytes and the hydrocortisone-resistant thymocytes were not agglutinated by PNA (Fig. 1). These findings are contrary to the

Abbreviations: D-RadLV, radiation leukemia virus variant; PNA, peanut agglutinin; P/NaCl, phosphate-buffered saline.
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commonly accepted notion that tumor cells are more agglutinable than are their normal counterparts (11, 12).

The agglutination properties of the preleukemic cells could not be examined directly because as yet there is no method for the isolation of these cells. We therefore fractionated bone marrow and spleen cells (among which preleukemic cells are the most abundant in D-RadLV-inoculated mice) by agglutination with PNA and tested their leukemogenic potential by using the transplantation bioassay method.

The same number (10^7) of unseparated, PNA+, and PNA- bone marrow cells collected from C57BL/6 mice previously inoculated with D-RadLV or P1/NaCl (the solution used for virus preparation) were injected intravenously into (BALB/c \( \times \) C57BL/6)F1 mice, 1–3 hr after they were exposed to 400 R (0.1 coulomb/kg) of whole-body irradiation (a nonleukemogenic dose that facilitates the proliferation of the injected cells).

In order to define whether the leukemias originated from the donor cells (C57BL/6), a genotype analysis was performed in which the leukemic cells developing in the F1 hybrid were transplanted into the parent strain. Tumors of donor C57BL/6 origin indicate the actual transfer of established preleukemic cells among the transferred cells, whereas tumors of (BALB/c \( \times \) C57BL/6)F1 host origin indicate transfer of a noncellular agent that caused neoplastic transformation of thymus-derived lymphoid cells of the irradiated F1 recipient. The results obtained (Table 1) clearly show that the preleukemic cells were present among the cells agglutinated by PNA. A high leukemia incidence (65%) of donor origin was obtained after transfer of preleukemic bone marrow cells agglutinated by PNA (an incidence similar to that obtained after transfer of unseparated preleukemic bone marrow—i.e., 80% leukemia induction—all of donor origin). This is in contrast to a low leukemia incidence (28%) of variable origin obtained after transplantation of unagglutinated preleukemic bone marrow cells and lack of preleukemic cells of donor origin among normal C57BL/6 bone marrow cells. Hybrid mice (15 per group) receiving unseparated splenocytes (from D-RadLV-treated donors) or splenocytes that were PNA- died within several weeks due to a graft-versus-host reaction. PNA+ splenocytes [shown previously to be devoid of cells inducing graft versus host activity (8)] which did not affect the life-span of the recipients contained preleukemic cells that ultimately inoculated a high leukemia incidence of donor origin (Table 1). The presence of preleukemic cells [shown previously to have the characteristics of prothymocytes (4)] mainly among PNA+ cells is in accordance with recent findings (13) that prothymocytes of mouse spleen are agglutinable by PNA.

Because the preleukemic cells are induced by intrathymic virus administration, it seemed of interest to define the thymus subpopulations susceptible to the leukemogenic transformation. Both in vivo and in vitro thymocyte–virus interactions were studied and the leukemogenic potential of the different PNA-separated populations was evaluated. The in vivo test involved intrathymic injection of D-RadLV. After 24 hr the thymocytes were separated into PNA+ and PNA- cells (7) and their ca-

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<th>Cell fraction tested</th>
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<td>Incidence</td>
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* Cells (10^7) were injected intravenously into (BALB/c \( \times \) C57BL/6)F1 mice (8–10 weeks old) within 1–3 hr after exposure to 400 R of whole-body irradiation. ALP, average latent period.
† Preleukemic cells were collected 30 days after D-RadLV intrathymic injections in C57BL/6 mice 6–8 weeks old (for details see ref. 1).
‡ Normal donors age matched to preleukemic donors.
§ D-RadLV was injected into the thymus of 6-week-old C57BL/6 mice; thymocytes were collected 24 hr later.
¶ D-RadLV was incubated with thymocytes (8:1) for 60 min at room temperature; the cells were washed three times before further transplantation.
Capacity to induce leukemia was tested by using the above mentioned transplantation bioassay method. The in vitro experiment involved separation of normal C57BL/6 thymocytes by PNA (7) and incubation of the PNA⁺ or PNA⁻ thymocytes with D-RadLV for 60 min at room temperature. After repeated washings (three times with 5 ml of P/NaCl), the cells were injected into F₁ irradiated recipients and leukemia induction in the recipients was observed. The results (Table 1) show that cells susceptible to D-RadLV transformation (yielding a majority of leukemias of donor origin) were present mainly among the PNA⁺ thymocytes.

Because PNA reacts preferentially with immature lymphocytes (7, 8, 13–16), our results provide additional evidence on the immature character of the preleukemic cells.

In conclusion, our findings demonstrate that the transformed cells present during the two distinct phases in D-RadLV leukemogenesis (5)—the dependent phase represented by preleukemic cells and the autonomous phase involving transition to leukemic cells—differ in their agglutinability by PNA.

This work was supported by Contracts NOI-CB-74163 (to N.S.) and NOI-CB-74151 (to N.H.) from the National Institutes of Health (U.S. Public Health Service). N.S. is an Established Investigator of the Chief Scientist's Bureau, Israel Ministry of Health.
