ONCOLOGY

Partial Elimination of Lymphocytes in Mice with Ehrlich Carcinoma

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Elimination of about 30% lymphocyte population was observed in female Balb/c mice on day 11 after transplantation of Ehrlich carcinoma in comparison with animals without tumor. It was hypothesized that the eliminated population can block the tumor growth. Studies of the temporal and quantitative parameters of lymphocyte elimination with consideration for tumor size are considered to be perspective. The described model can be used for studies of immunodeficiency in animals with tumors.

Key Words: *immunodeficiency; experimental tumors*

The hypothesis that tumor growth in associated with the development of immunodeficiency seems to be very logical at first sight. Surprisingly, no manifest disorders in the immune system were detected in the majority of cancer patients (except those with lymphoproliferative diseases) [3,4,6,7].

We previously described metastasizing of Ehrlich carcinoma in mice after surgical removal of the primary tumor [1]; one of the explanations was immunodeficiency in animals with tumors. Here we used this model for investigating possible immunodeficiency during the development of tumor process with the minimum tumor volume in the absence of apparent changes in differential leukocyte count [2].

MATERIALS AND METHODS

Female Balb/c mice aged 2-3 months were used in the study. Ehrlich mammary carcinoma cells were transplanted intramuscularly (10^6 cells in 0.2 ml RPMI-1640). Lymphocytes were isolated from the peripheral blood on day 7 posttransplantation by centrifugation in a 1.093 density gradient.

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Polyclonal rabbit antibodies to lymphocytes of tumor-bearing mice were obtained from the sera of immunized animals by protein G affinity chromatography. Fab fragments of antilymphocytic antibodies were obtained and labeled with FITC in order to minimize nonspecific antibody binding to the surface of mouse lymphocytes.

Similarly, serum antibodies from a nonimmune rabbit were isolated and Fab fragments were obtained and labeled with FITC, which were then used for evaluating nonspecific reactions with mouse lymphocytes. The cells were incubated with labeled Fab fragments at 4°C for 1 h, washed by centrifugation, and fixed in 0.5% paraform solution. Cell fluorescence was measured on a Calibur flow cytofluorometer.

RESULTS

All lymphocytes from mice with Ehrlich carcinoma after incubation with FITC-labeled Fab fragments of immunoglobulins to these lymphocytes were stained with FITC and formed a single wide peak (Fig. 1, *c*). The width of the peak could be due to great heterogeneity of the antibody Fab fragments used in the experiment. Almost 92% cells were stained.

Reaction of Fab fragments of antibodies against lymphocytes from tumor-bearing mice with lympho-

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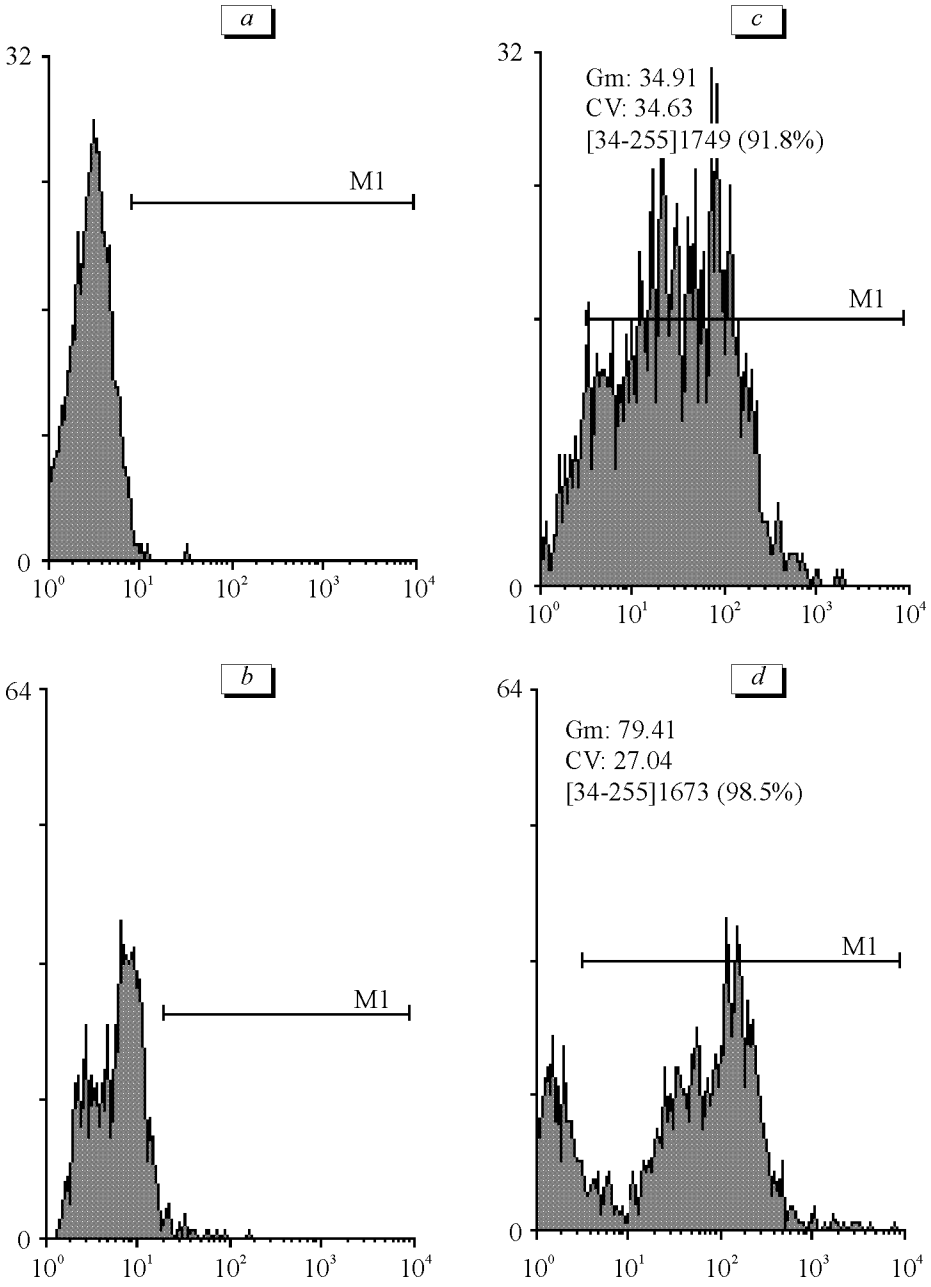

Fig. 1. Histograms of peripheral blood lymphocyte populations in mice with Ehrlich carcinoma (*a*-*c*) and without tumor (*d*). *a*) initial population; *b*) after incubation with Fab fragments of nonimmune antibodies; *c*, *d*) with Fab fragments of antibodies to lymphocytes of mice with Ehrlich carcinoma.

cytes from mice without tumors revealed a new population of lymphocytes (20% unstained cells) and decreased percentage of stained cells (Fig. 1, *d*). The appearance of unstained lymphocyte population indicates that some cells present in intact animals were absent in the lymphocyte population of mice with tumors. The position of this population on histograms corresponds to the position of lymphocytes incubated with Fab fragments of nonimmune antibodies (Fig. 1, *b*). Fab fragments of nonimmune immunoglobulins did not intensely stain the peripheral blood lymphocytes in mice with tumors (only 2.2%). However the minimum adsorption of these Fab fragments on lymphocytes manifested in a slight cleavage of the peak, which was absent in the initial lymphocyte population. Hence, changes in control histograms attest to the presence of a lymphocyte population disappearing during the development of tumor process.

We consider that these results can be explained by elimination of an appreciable portion (about 30%) of lymphocytes with certain antigenic determinants in mice with tumors, and therefore antibodies which might react with these cells are absent. For comparison, *in vivo* lymphocyte activation with various lymphokines yields just tenths of percent activated lymphocytes [5]. It should be emphasized that the first changes in lymphocyte population manifest as early as on day 3 after tumor transplantation, *i.e.* before the appearance of a

visible tumor. Presumably, eliminated lymphocytes can block tumor growth. This explains why changes in the immune system of cancer patients are rare (some cell clones are eliminated, but the ratio of lymphocyte subpopulations remains unchanged) and why the effects of antitumor vaccines are short-lasting (elimination of lymphocyte clones capable of reacting to these antigens). The study of the temporal and quantitative parameters of lymphocyte elimination in tumors of different volumes seems to be an interesting problem. The model described in this paper can be used for studies of immunodeficiency in animals with tumors.

REFERENCES

1. F. V. Donenko, S. M. Sitdikova, A. O. Kabieva, and L. V. Moroz, *Byull. Eksp. Biol. Med.*, **114**, No. 12, 652-654 (1992).
2. F. V. Donenko, S. M. Sitdikova, A. O. Kabieva, and L. V. Moroz, *Ibid.*, **124**, No. 10, 443-445 (1997).
3. M. E. Boon, G. van der Veen, Y. Barlow, *et al.*, *Diagn. Cytopathol.*, **24**, No. 6, 373-377 (2001).
4. M. Frisch, R. J. Biggar, and J. J. Goedert, *J. Natl. Cancer Inst.*, **92**, No. 18, 1500-1510 (2000).
5. H. T. Maecker, H. S. Dunn, M. A. Suni, *et al.*, *J. Immunol. Meth.*, **255**, 27-40 (2001).
6. G. J. Maestroni, *Adv. Exp. Med. Biol.*, **467**, 217-226 (1999).
7. G. Vartholomatos, E. Kapsali, E. Dokou, and K. L. Bourantas, *J. Exp. Clin. Cancer Res.*, **20**, No. 2, 305-306 (2001).