101. Protection against Epstein-Barr Virus Oncogenesis

Cooperative Effect of Lymphocytes, Antibodies and Interferon*

By Takao Aya, Toyoro Osato, and Tsunataro Kishida**


Introduction. Epstein-Barr virus (EBV), which is highly suspected to have a causal relationship to Burkitt lymphoma and nasopharyngeal carcinoma, infects most humans without producing any serious disease (cf. Epstein and Achong, 1979). Consequently, there has been a strong argument for an important role for immunologic surveillance in the protection against EBV-induced oncogenesis (cf. Epstein and Achong, 1979), and our previous investigations have suggested that antibody-dependent cellular cytotoxicity (ADCC) plays a major role among several possible immunologic mechanisms (Aya et al., 1978, 1980).

The present paper describes a finding showing that on the basis of ADCC more effective protection against EBV infection and oncogenesis has been noted by addition of a small amount of human interferon.

Materials and methods. Peripheral lymphocytes from EBV-seropositive healthy donors were converted in vitro into lymphoblasts by exposure to the B95-8 EBV (Miller and Lipman, 1973). The blast cells were used in 1–2 months after establishment of cell lines as freshly EBV-transformed target cells. On the other hand, lymphocytes were exposed to EBV and immediately treated with trypsin to remove cell-bound residual virus (Sairenji et al., 1978). The newly infected lymphocytes also served as target cells. All the cultures were grown in the RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum.

The effector cells were autologous lymphocytes corresponding to donor target cells. These lymphocytes were obtained from heparinized peripheral blood by centrifugation on Isopaque-Ficoll gradients. Macrophages were removed by the silica technique (Tada et al., 1980).

For EBV antibodies, standard seropositive serum γ-globulin and

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* Address inquiries to: Dr. Toyoro Osato, Department of Virology, Cancer Institute, Hokkaido University School of Medicine, Sapporo 060, Japan.

** Department of Virology, Cancer Institute, Hokkaido University School of Medicine, Sapporo 060.

*** Department of Microbiology, Kyoto Prefectural University of Medicine, Kyoto 602.
serum from each seropositive donor were used. Seronegative γ-globulin served as a control. These sera were heated at 56°C for 30 min and examined for antibody titers to EBV-related antigens. The titer to the membrane antigens (MA) (Klein et al. 1966) was most important (Aya et al., 1978, 1980).

Interferon preparations were made by one of authors (T.K.). Human leukocyte interferon was induced by inoculation with Sendai virus, concentrated and partially purified, as previously described (Matsuo et al., 1974). The specific activity was $5.0 \times 10^6$ international units (IU)/mg protein. As controls, mouse brain interferon induced by Japanese B encephalitis virus (specific activity $5.0 \times 10^5$ IU/mg protein), and heated (70°C, 10 min) human interferon were used.

Cytotoxicity was tested by the specific trypan blue exclusion of target cells, as described previously (Aya et al., 1978, 1980). Briefly, viable cells were first stained with trypan blue, smeared, dried, fixed and then stained according to the anticomplement method for EBNA (Reedman and Klein, 1973). The immunofluorescence and the trypan blue exclusion were simultaneously observed in the same microscopic field under a Leitz Orthoplan, by changing UV illumination to visible light. Cytotoxicity was calculated by the following formula: 

$$\frac{\text{No. of trypan blue-stained EBNA-positive cells}}{\text{No. of EBNA-positive cells}} \times 100.$$ 

**Results.** High frequency of cytotoxicity against EBV-transformed cells in the presence of lymphocytes, antibodies and interferon. When freshly EBV-transformed autologous lymphocytes were exposed simultaneously to donor peripheral lymphocytes, EBV-seropositive serum and human interferon, the transformed cells were killed at high frequency, as judged by the trypan blue-EBNA double staining method. The cytotoxicity by the mixture of lymphocytes and antibodies, i.e. ADCC, which was a major immunologic protection mechanism against EBV-induced transformation (Aya et al., 1978, 1980), was enhanced several-fold by addition of a small amount of interferon. In contrast to this, the transformed cells were not significantly killed by incubation with either lymphocytes alone, EBV-seropositive serum alone, or even with a much larger dose of interferon alone. The transformed target cells were also not damaged significantly by treatment with an autologous lymphocyte-interferon mixture. The data are illustrated in Fig. 1. No cytotoxicity enhancement was remarkable under the effect of mouse interferon or heated human interferon.

Remarkable growth inhibition of newly EBV-infected cells in the presence of lymphocytes, antibodies and interferon. It was then examined whether or not the growth of newly EBV-infected lym-
phocytes would be inhibited markedly under the same conditions as described above. In experiment, one of 200 lymphocytes were actually infected by the given EBV inoculum, indicating that the ratio of effector lymphocytes to target cells was 200:1 (Aya et al., 1978, 1980).

After treatment with trypsin, the lymphocytes exposed to EBV were immediately incubated with the medium containing seropositive serum and/or human interferon for several days. As shown in Fig. 2, the growth of the EBNA-positive lymphocytes was significantly inhibited in the presence of uninfected lymphocytes and EBV-seropositive serum, as described previously (Aya et al., 1978, 1980). In addition, when a very small amount of interferon was also present, a remarkable enhancement of the growth inhibition occurred. No significant inhibition was noted by exposure to a much larger dose of interferon (1,000 IU) alone (data not shown).

Discussion. As an in vitro model of immunologic surveillance
of EBV-induced oncogenesis, our previous investigations have indicated in an autologous system that ADCC is probably the most effective mechanism among several possible immunologic mechanisms (Aya et al., 1978, 1980).

The present findings suggest further that this EBV-related ADCC is much enhanced by adding a small amount of human interferon. Interferon alone, however, was not cytoxic enough, even though a much larger dose was given. Since the mixture of lymphocytes and interferon was not significantly effective, the high frequency of cytotoxicity in this study does not seem to be related to the enhancement of natural killer (NK) cell activity as generally known (Einhorn et al., 1978; Trinchieri and Santoli, 1978; Skurkovich et al., 1978; Sato et al., 1979; Zarling et al., 1979; Herberman et al., 1979; Droller et al., 1979; Saksela et al., 1979; Moore and Potter, 1980). A similar type of enhancement of ADCC by interferon was also suggested recently in a different experimental system other than EBV (Herberman et al., 1979; Droller et al., 1979).

Fig. 2. Remarkable growth inhibition of newly EBV-infected cells by simultaneous exposure to EBV antibodies, uninfected lymphocytes (see text), and a small amount of human interferon. ×—×, EBV-infected lymphocytes (control). ○—○, EBV-infected lymphocytes in the presence of seropositive γ-globulin (MA titer of 1:160). •—•, EBV-infected lymphocytes in the presence of seropositive γ-globulin and interferon (5 IU).
EBV is widespread in man with antibody response, and a small amount of interferon is possibly present normally in the body. It is therefore conceivable that the cooperation of lymphocytes, EBV antibodies and interferon plays a most effective role in the protection against EBV-induced oncogenesis in seropositive healthy individuals.

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