THE CLINICAL SIGNIFICANCE OF IMMUNOLOGICAL FINDINGS IN BURKITT'S LYMPHOMA

(EPSTEIN-BARR VIRUS-ASSOCIATED ANTIBODIES/ BCG TREATMENT)

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Abbreviations: BL = Burkitt's lymphoma; EA = EBV-induced early antigen complex, divided into D ("diffuse") and R ("restricted") subcomponents; EBV = Epstein-Barr virus; MA = EBV-associated cell membrane antigen complex; PDT = pertussis-diphtheria-tetanus triple vaccine; VCA = EB viral capsid antigen complex.

Abstract

All Burkitt's lymphoma patients studied have had antibodies against certain Epstein-Barr virus-associated antigens, in contrast to control persons who sometimes lack such detectable antibodies. High titers of one type of Epstein-Barr virus-associated antibody correlated to a greater risk for recurrences and death than low titers, and another virus associated antibody was sometimes noted to decrease significantly before late tumor recurrences. BCG inoculations regularly increased the latter antibody titers.

BL occurs with time and space clustering, suggesting epidemiological factors in its etiology. Long term survival after few and relatively low doses of cytostatics, and occasional spontaneous regressions have been noted. These circumstances made immunologists and virologists investigate the disease, resulting in the demonstration of EBV-associated antigens: MA on tumor cells and derived lines by KLEIN *et al.* (1), VCA in tumor-derived lines by HENLE and HENLE (2), and EA in recently EBV-superinfected lymphoblastoid lines (3). EA was further divided into R and D components (4).

Every BL patient studied has had antibodies to MA (5) and these antibodies decreased suddenly 1/2 year before a recurrence after 4 years tumor regression in one patient (6). Control persons did not have the same high incidence of high anti-MA levels as BL patients, though some control subjects had antibody levels comparable to those of most BL cases. Similar findings were obtained with regard to anti-VCA, but these antibodies were not clearly influenced by clinical events (7). High anti-EA titers correlated to a greater risk for recurrences and death than low titers (8) and the significant antibody usually seemed to be anti-R (9).

We have followed BL patients horizontally by antibody titrations during the course of their disease to see whether titer changes occurred in relation to clinical events.

Material and methods

BL patients' sera were tested for anti-MA by their blocking of the direct membrane immunofluorescence reaction between a BL-derived IgG and BL-derived cultured cells expressing MA (10). Blocking activity was measured by a blocking index, ranging from 0.00 to 1.00. The index fell after serum dilution and a titer could be calculated with titer endpoint = index 0.4 (11). Anti-VCA and anti-EA were tested by indirect immunofluorescence, using serum dilutions on fixed smears of antigen-positive cells (2, 7, 3, 4).

Histologically verified BL patients that became clinically tumor free after chemotherapy were given up to 10 intradermal applications of BCG (Glaxo Ltd) by a Heaf gun, usually with 3 weeks intervals. The procedure was described in detail (12). Some patients received one i. m. injection of 0.5 ml pertussis-diphteria-tetanus vaccine ("Trivax", Burroughs Wellcome and Co.) in exchange for one of the later BCG inoculations.

Results

A study of 5 BL patients (12) showed that anti-MA titers usually were stable during chemotherapy and tumor regression. A slight titer peak was noted in time relation to recurrences in 3 patients and larger peaks in all patients following BCG inoculations. The latter increases had a maximal size of 1–2 $\log_2$ units but usually faded during repeated BCG administrations. Prolonged tumor-free survival did not change these titers significantly, as a rule.

Anti-EA sometimes started to increase before clinical recognition of recurrent tumors. In most patients these titers decreased slowly during prolonged remission, even during BCG treatment.

Anti-VCA usually showed an increase during the first few months after admission and sometimes peaks in time relation to recurrent tumors. No significant changes were seen during BCG treatment and prolonged survival.

The observed fading of anti-MA titer peaks during repeated BCG administrations was speculated to follow an increasing host immunisation to BCG, that would limit its effect as a non-specific immune adjuvant. For this reason one of the later BCG inoculations was exchanged for PDT in some patients. The first one of these had widespread recurrences closely after the PDT vaccination. A serologic analysis showed that anti-MA titers decreased significantly well before PDT was given, accompanied by rising anti-EA (13). These findings indicated that the host-tumor balance had changed already before PDT vaccination.

Discussion

EBV-associated antibody titers were found to sometimes change significantly before recurrences and during non-specific immune stimulant therapy in BL patients. The mechanisms responsible for these changes remain largely unknown.

BL biopsy cells have MA but no detectable EA or VCA (14), and this may explain the unique behavior of anti-MA titers during BCG inoculation periods. VCA synthesis must occur somewhere, however, to maintain the high anti-VCA titers observed during prolonged tumorfree survival. The simultaneous decrease of anti-EA seen in some patients could be due to lack of antigenic stimulation during sustained remission.

The clinical value of BCG treatment in BL patients is still unknown in the absence of an adequate control group for the patients in this pilot study.

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