Antigenic Differences between the Membrane Antigen Polypeptides Determined by Different EB Virus Isolates

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SUMMARY

The antigenic specificities of the membrane antigen (MA) complex on three Epstein-Barr (EB) virus-producing cell lines have been compared by complete absorption of an anti-MA antiserum with P3HR-1 cells, followed by testing for residual anti-MA antibody activity against B95-8 and QIMR-WIL cells. Indirect membrane immunofluorescence showed that the absorbed serum, which had lost the capacity to bind P3HR-1 cells, nevertheless gave bright staining on a small proportion of B95-8 cells. SDS-polyacrylamide gel electrophoretic analysis of 125I-labelled MA polypeptides showed that the absorbed serum was able to isolate MA polypeptides from both B95-8 and QIMR-WIL cells, but not from P3HR-1 cells. The results demonstrate that there is substantial sharing of antigenic specificities between the MA determined by three isolates of EB virus, and some antigenic divergence.

Cultures of human or marmoset lymphoid cell lines transformed with Epstein-Barr (EB) virus, contain variable proportions of cells which have entered the late phase of virus replication and which display EB virus-determined membrane antigens (MA) (Klein et al., 1969). MA was shown to be a complex of antigenic specificities by Svedmyr et al. (1970), and this observation is substantiated by a number of investigations which have identified four glycosylated polypeptides in the MA complex (Qualtière & Pearson, 1979; Thorley-Lawson & Edson, 1979; North et al., 1980). Furthermore, recent studies (North et al., 1980) have demonstrated that the polypeptide components of cell surface MA are also found on the envelope of mature EB virus particles, thereby providing a molecular basis for the explanation of previous observations that some virus-neutralizing antibodies in human serum could be absorbed out by exposure to MA-positive cell lines (Pearson et al., 1971; Gergely et al., 1971), that antibodies to MA bound to virus particles (Silvestre et al., 1974), and that sera with anti-MA activity often showed virus-neutralizing capacity (Pearson et al., 1970, 1971; Gergely et al., 1971; de Schryver et al., 1974).

Variations in both mol. wt. and relative abundance of the two largest polypeptides of the MA complex have been observed when MA displayed on different cell lines has been compared (Thorley-Lawson & Edson, 1979; Qualtière & Pearson, 1979, 1980; North et al., 1980). The mol. wt. reported by different laboratories differ slightly, but there is general agreement that EB virus-producing marmoset cell lines carrying the B95-8 isolate display a dominant MA component with mol. wt. of about 340000 and a minor component of mol. wt. 270000. In contrast, human cell lines superinfected with, or producing the P3HR-1 isolate of EB virus have a minor component of 320000, the major polypeptide being of 240000 mol. wt. Although the mol. wt. variations (340000 versus 320000 and 270000 versus 240000) appear to correlate with the species of origin of the cells carrying the virus (North et al., 1980) and may therefore arise from differences in host cell-directed glycosylation of virus-specified polypeptides, the significance of the differences in the amounts of these highest mol. wt. MA components in different cell lines is less clear. However, since B95-8 and P3HR-1 EB virus differ both in transforming ability (Miller et al., 1974) and in their DNA organization and
sequence (Bornkamm et al., 1980), it is natural to speculate that these differences in MA reflect genetic variations.

In view of the observed mol. wt. differences, we have investigated whether antigenic differences exist between the MA molecules displayed by three EB virus-producing cell lines: P3HR-1, a human cell line derived from a case of Burkitt's lymphoma (Jijoye) (Henle et al., 1967)—it should be noted that after these cells were cloned, they produced virus which had lost the transforming ability (Miller et al., 1974) characteristic of all other EB virus isolates; B95-8, a marmoset lymphoblastoid cell line established using virus from an individual with perfusion mononucleosis (Miller et al., 1972); and QIMR-WIL, a human lymphoblastoid cell line of the type obtainable from any individual seropositive for EB virus, although the donor suffered from myeloblastic leukaemia (Pope, 1968). The present communication describes experiments in which serum (HS18) from a normal human MA-seropositive donor (kindly provided by the Southwest Regional Blood Transfusion Centre, Bristol, U.K.) was absorbed with P3HR-1 cells until all subsequent indirect membrane immunofluorescence with these was abolished; after this, the absorbed serum was tested for indirect membrane immunofluorescence on B95-8 cells, and for the ability to isolate $^{125}$I-labelled MA polypeptides from B95-8 and QIMR-WIL cells. A monoclonal antibody designated 72A1 (generously made available by Dr G. J. Hoffman, Johns Hopkins School of Medicine, Baltimore, U.S.A.) (Hoffman et al., 1980) has also been used to isolate $^{125}$I-labelled MA polypeptides, while a seronegative human serum (HS15) served as a control.

Details of the methods of cell culture, cell surface radioiodination using Na$^{125}$I and lactoperoxidase, immune complex isolation and SDS–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of $^{125}$I-labelled MA polypeptides, have been described in full elsewhere (North et al., 1980). Mol. wt. standards used in SDS–PAGE were purchased from Pharmacia: thyroglobulin (330000), ferritin (220000), phosphorylase b (94000), bovine serum albumin (67000) and trypsinogen (24000). Indirect membrane immunofluorescence tests were carried out following established procedures (Klein et al., 1967) using a FITC conjugate of sheep anti-human IgG (Wellcome Reagents).

HS18 serum was absorbed repeatedly with P3HR-1 cells at a ratio of $10^9$ cells/ml serum diluted 1:3. For each absorption, a pellet of washed cells was resuspended in the appropriate volume of diluted serum and incubated with gentle agitation for 30 min at 20 °C, followed by 2 h at 4 °C. Cells were removed by centrifugation at 350 g for 10 min. and the absorbed serum clarified by centrifugation at 30000 g for 30 min. After each absorption, the serum was tested by indirect immunofluorescence for its ability to give membrane staining of P3HR-1 cells, and no further MA staining on P3HR-1 cells could be detected following seven sequential absorptions.

In previous experiments using indirect membrane immunofluorescence, it was observed that P3HR-1 and QIMR-WIL cells showed MA fluorescence as two to four bright patches/positive cell. In contrast, cells from B95-8 cultures showed either one to 30 weakly fluorescent spots or very bright staining over the whole plasmalemma. The first type of staining involved between 10 and 75% of the population and the second type, from 1 to 5% of the population; the more virus produced by a culture, the greater the number of cells showing each staining pattern. All the staining was readily distinguishable from the fluorescence of dead cells.

When HS18 serum was used after absorption with P3HR-1 cells to stain B95-8 cells, the indirect immunofluorescence of the cells showing spot staining was markedly reduced (Fig. 1a), whereas that of the small proportion of cells with staining over the whole surface remained essentially unchanged (Fig. 1b). The results suggest that the cells of B95-8 cultures showing both full surface fluorescence and, to a lesser extent, the spot staining, have antigenic specificities not found on P3HR-1 cells.
Fig. 1. Indirect membrane immunofluorescence on B95-8 cells using human seropositive serum HS 18 before (filled symbols) and after (open symbols) absorption with P3HR-1 cells. (a) Spot fluorescence: three experiments in which the maximum proportion of cells with fluorescent spots (shown as 100%) was 20% (●, ◦), 74% (▲, △) and 14% (■, □) of the population. (b) Full surface fluorescence: data from the same three experiments, in which the maximum proportion of cells with full surface fluorescence (normalized to 2%) was 2%, 3% and 3% respectively, have been combined to give geometric means. Error bars indicate two standard deviations for each point. Numbers in parentheses indicate the total number of cells with full surface fluorescence scored for each point. The differences observed between absorbed (●) and unabsorbed (○) HS 18 serum are not significant.

To determine whether this antigenic difference between the MA complex of P3HR-1 and B95-8 cells was related to the previously established differences in the mol. wt. of the MA polypeptides from these sources (North et al., 1980), the absorbed serum was tested for its ability to isolate MA polypeptides from each of three cell lines. Tracks A and J of Fig. 2 show that as previously reported (North et al., 1980), HS18 serum binds three components of MA from P3HR-1 and QIMR-WIL cells. By contrast, the absorbed HS18 serum was unable to isolate any of the three major MA components of P3HR-1 (Fig. 2, track C), thus confirming the findings from the indirect immunofluorescence tests. Nevertheless, the absorbed serum was able to isolate a small proportion of the 340000 mol. wt. polypeptide from a B95-8 lysate (Fig. 2, tracks D and G) and of both the 320000 and 240000 mol. wt. polypeptides from a QIMR-WIL lysate (Fig. 2, track L). An 85 000 mol. wt. component of MA isolated from P3HR-1, QIMR-WIL and B95-8 cell lysates with HS18 serum, was not isolated from P3HR-1 or B95-8 lysates after absorption of the serum. However, the absorbed serum did isolate a proportion of the 85 000 mol. wt. MA polypeptides from QIMR-WIL (Fig. 2, track L). Thus, no antigenic difference was detected between the 85 000 mol. wt. components of P3HR-1 and B95-8 MA, although the QIMR-WIL 85 000 mol. wt. MA polypeptide possesses antigens not found on P3HR-1 cells.

Absorption of much of the antibody to the 340 000 mol. wt. component of B95-8 MA by P3HR-1 cells displaying predominantly a 240 000 mol. wt. polypeptide, suggests substantial sharing of antigenic determinants between these molecules. The presence of common antigens is unequivocally demonstrated by the ability of a monoclonal antibody, derived by cell fusion techniques from mice immunized with B95-8 EB virus (Hoffman et al., 1980), to isolate both 320 000 and 240 000 mol. wt. MA components from P3HR-1 cells (Fig. 2, track M) as well as a high mol. wt. polypeptide from B95-8 cells (track N). The electrophoretic mobility of the MA component isolated in this laboratory from B95-8 cells using 72A1 monoclonal antibody is indistinguishable from that isolated using human seropositive serum HS18, giving a mol. wt.
Fig. 2. Autoradiographs of SDS-PAGE showing $^{125}$I-labelled MA polypeptides isolated after Triton X-100 lysis of cells from three cell lines. The antisera used for MA isolation were: tracks, A, F, I and J, unabsorbed HS18 serum (anti-MA+); tracks B, E, H and K, unabsorbed HS15 serum (anti-MA-); tracks C, D, G and L, HS18 serum after absorption with P3HR-1 cells; tracks M and N, monoclonal antibody 72A1. Numbers indicate apparent mol. wt. ($\times 10^{-3}$) of MA polypeptides, estimated from their mobilities relative to marker proteins of known mol. wt. (markers not shown). It should be noted that with the particular samples of cultures used for these experiments, the B95-8 cells expressed the 270 000 mol. wt. MA component rather weakly, and in consequence it does not show up well on the autoradiographs (tracks F, I and N).

of 340 000 (North et al., 1980). It is likely that the B95-8 MA polypeptide observed by Hoffman et al. (1980) using this antibody (put at 250 000 mol. wt.) is identical to the 340 000 mol. wt. MA species described here, the absence of a mol. wt. standard above 220 000 contributing to the lower mol. wt. quoted (Hoffman et al., 1980). Two other monoclonal antibodies to MA also isolate both of the high mol. wt. MA polypeptides from QIMR-WIL, B95-8 and P3HR-1 cells (Thorley-Lawson & Geilinger, 1980; J. R. North, unpublished data using the antibody of Thorley-Lawson & Geilinger, 1980). It is therefore apparent that the two high mol. wt. MA polypeptides on each cell line are structurally related both to one another and to the equivalent components in the MA of the other EB virus-producing cell lines tested. Nevertheless, the incomplete absorption of antibodies to B95-8 and QIMR-WIL MA by P3HR-1 cells indicates antigenic divergence between the MA determined by these particular isolates of EB virus.

MA components are known to be glycoproteins (Thorley-Lawson & Edson, 1979; Qualtière & Pearson, 1979, 1980; Strnad et al., 1979), but it is at present unclear whether the antigenic differences detected here result from alterations in the polypeptide or carbohydrate portions of the molecules. Although the species of origin of the host cell appears to contribute to the mol. wt. of EB virus-determined MA polypeptides (North et al., 1980), other factors must influence the antigenic difference since both P3HR-1 and QIMR-WIL cells are of human origin.

Although there is as yet no biological, immunological or biochemical evidence for disease-related strain differences amongst EB virus isolates (Kawai et al., 1973; Nonoyama &
Pagano, 1973; Kieff & Levine, 1974; Menezes et al., 1975; Miller et al., 1976; Pagano et al., 1976; Gerber et al., 1976; Fialkow, 1976; Kaschka-Dierich et al., 1977; Sugden, 1977; Adams et al., 1979; Rymo et al., 1979; Bornkamm et al., 1980), it has long been known that the P3HR-1 and B95-8 strains are anomalous. Thus, P3HR-1 EB virus is the only strain known to have lost transforming ability (Gerber et al., 1969; Miller et al., 1974) and to have marked perturbations of genome structure (Raab-Traub et al., 1978; Bornkamm et al., 1980), whilst the B95-8 strain of the virus lacks at least 10% of the usual viral DNA (Raab-Traub et al., 1978; Delius & Bornkamm, 1978; Given & Kieff, 1978; Bornkamm et al., 1980).

If the antigenic variation in MA reported here is not in fact due to differences in host cell-directed glycosylation of virus-determined polypeptides, it seems probable that the variation may reflect one aspect of the differences in DNA size and organization known to occur amongst anomalous strains of EB virus.

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REFERENCES


Short communications


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