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Effect of the Arginine Analog Canavanine on the Synthesis of Epstein-Barr Virus-Induced Proteins in Superinfected Raji Cells

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The addition of canavanine to cultures of superinfected Raji cells in the absence of arginine prevented the appearance of early antigens as defined by immunofluorescent staining. Addition of various amounts of arginine permitted the identification of at least three groups of proteins, each responding differently to the various concentrations of arginine-canavanine.

Superinfection of Raji cells with Epstein-Barr virus (EBV) derived from P3HR1 cells (HR1-EBV) leads to the synthesis of a variety of virus-induced proteins which can be divided into several groups according to their kinetics of synthesis, their requirement for viral DNA synthesis response to cycloheximide blocks applied at different times after infection, and their synthesis in the presence of amino acid analogs (1, 1a, 2, 4–6, 8). Previous studies by us indicated that treatment of superinfected Raji cells with canavanine in the absence of arginine prevented the synthesis of EBV early antigens (EA). Only a minimal expression of the virus could be observed; only three virus-induced polypeptides (120, 102, and 49) were identified in extracts of such cells. Treatment with azetidine (an analog of proline) did not have this drastic effect since the cells proceeded to EA synthesis but not virus capsid antigen (VCA) synthesis. A recent report (9) suggested that canavanine did not suppress the synthesis of EA in superinfected Raji cells.

Since the control of synthesis of early or intermediate early proteins in cells infected with oncogenic viruses can be seen as one of the factors that will determine whether the cell will become transformed or enter a lytic cycle, we investigated this apparent discrepancy. First we asked the question why canavanine has such a strong effect whereas azetidine is much less effective. Since it was shown that the synthesis of certain herpes simplex virus proteins could be affected by varying the arginine content of the culture medium (3, 7), we investigated the behavior of superinfected Raji cells under conditions of arginine starvation.

Raji cells were superinfected using a virus stock that had been diluted with arginine-free medium. After 1 h of adsorption the cells were washed with arginine-free medium twice and resuspended in either arginine-free medium or medium containing the normal amount of arginine. After 12 h the cells were labeled with [^{32}S]methionine in arginine-free or -containing medium. Figure 1B illustrates the results of such an experiment; S tracks are superinfected cells, and tracks indicated with a − are mock-infected controls. There was a slight overall reduction in protein synthesis in the absence of arginine, but all of the virus proteins could be identified. In a parallel set of cultures we included canavanine at a concentration of 0.9 mM, with arginine present at 0, 1, 10, or 100% of its normal concentration (0.95 mM) (Fig. 1A). As can be seen from this experiment, the EBV-induced proteins fall into at least three groups: (i) those synthesized in the presence of 0.9 mM canavanine regardless of the arginine concentration (e.g., protein 138); (ii) those proteins synthesized in the presence of 0.9 mM canavanine when arginine is present at 10 or 100% of its normal concentration (e.g., protein 150); and (iii) those proteins synthesized only when canavanine is omitted from the culture medium (e.g., protein 143).

In this experiment all superinfected cultures were positive for EBV EA. Our previous study (1a) suggested that EA was not synthesized in the presence of 2.7 mM canavanine in the absence of arginine. To confirm this finding we superinfected Raji cells in the absence of arginine and then incubated the cells for 12 h in media containing 0, 0.1, 0.3, 0.9, or 2.7 mM canavanine and 0, 1, 10, or 100% of the normal arginine concentration. After 12 h the cells were stained for EA and processed for analysis on polyacrylamide gels (Fig. 1D) or for immunoprecipitation (Fig. 1C). When superinfected Raji cells were incubated in the presence of 2.7 mM canavanine in media with 0 or 1% of the normal arginine concentration they remained negative

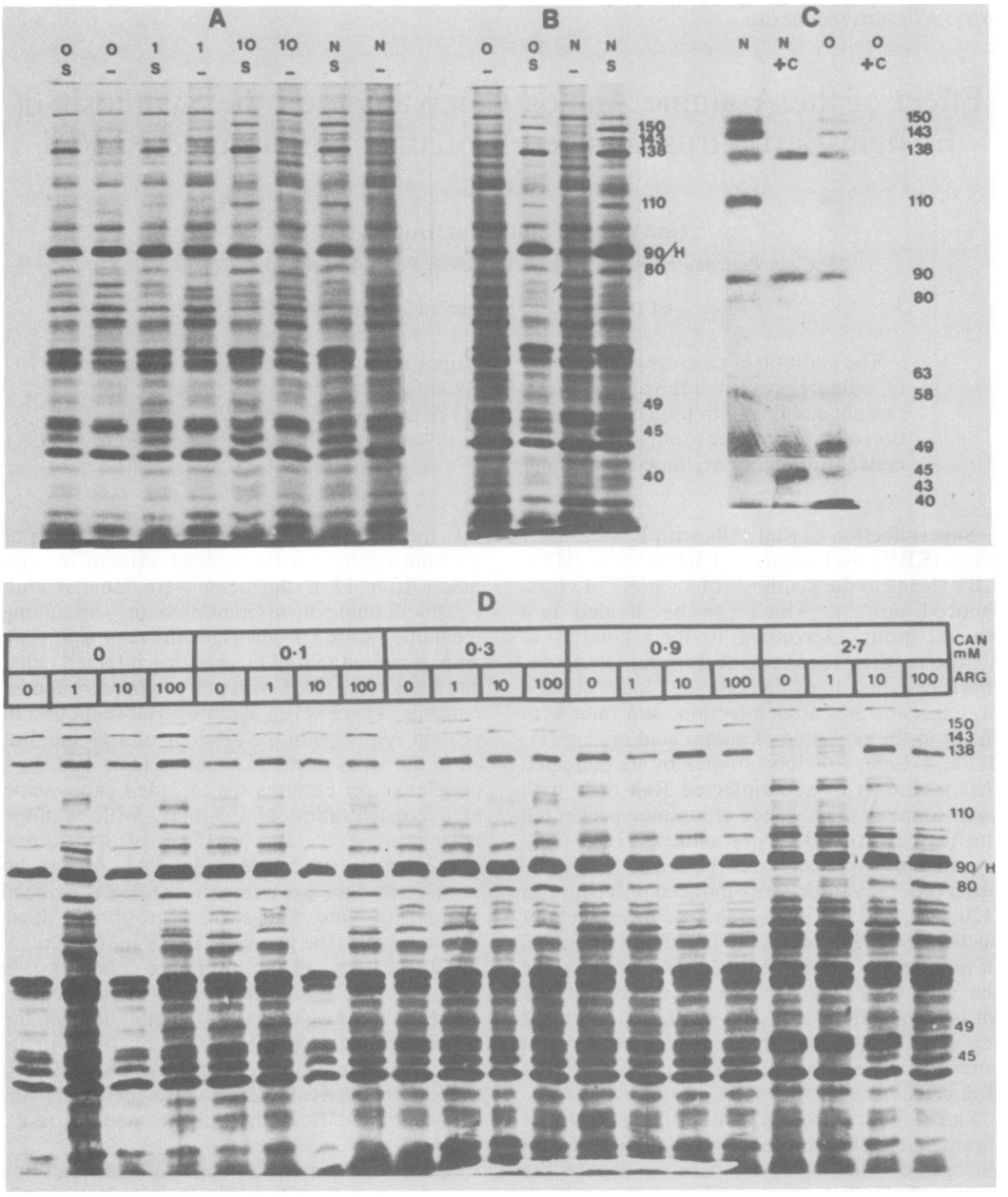

FIG. 1. Polyacrylamide gel analysis of EBV-induced proteins synthesized in the presence of varying amounts of canavanine and arginine. The virus-induced proteins are labeled with their approximate molecular weights. Protein 90/H is a complex band containing both virus and host proteins. (A and B) Raji cells were superinfected (S) or mock-infected (−) with HR1-EBV in the presence of 0.9 mM canavanine (A) or the absence of canavanine (B). Arginine was present at 0, 1, 10, or 100% of its normal concentration (tracks 0, 1, 10, and N, respectively). After adsorption the cultures were incubated for 12 h in media with the relevant arginine and canavanine concentrations. The cells were then pulse-labeled (30 min) with [^{35}S]methionine (50 μCi/ml) in methionine-free medium containing the relevant concentrations of canavanine and arginine. The cells were disrupted in electrophoresis sample buffer and analyzed on 10% polyacrylamide gels cross-linked with diallyltartardiamide containing 2% sodium dodecyl sulfate (for method details, see reference 1a). (C) Raji cells were superinfected in the presence (+C) or absence of 2.7 mM canavanine; arginine was either absent (0) or present at its normal concentration (N). The cells were incubated for 12 h, labeled as described for (A) and (B) for 4 h and then prepared for immunoprecipitation (as described in reference 1a). The immunoprecipitates were analyzed on polyacrylamide gels as described. (D) Raji cells were superinfected in the presence of canavanine (0, 0.1, 0.3, 0.7, and 2.7 mM) and various concentrations of arginine (0, 1, 10 or 100% of its normal concentration). At 12 h after infection the cells were processed as described for (A) and (B).

for EA; all other cultures were positive for this antigen complex. The immune precipitation revealed that at 0% arginine and 2.7 mM canavanine no immune precipitable proteins were synthesized (Fig. 1C, track 0 + C), whereas those cultures positive for EA contained a variety of immune precipitable EBV-induced proteins (Fig. 1C, tracks N, N + C, and 0). The treated and untreated mock controls did not contain any identifiable proteins (data not shown). In Fig. 1D the direct polyacrylamide gel electrophoretic analysis of all the cultures confirms this result in that at 2.7 mM canavanine in the presence of 0 or 1% of the normal arginine concentration none of the usual proteins could be identified. A number of faint bands not present in the mock-infected controls were identified; however, they cannot be convincingly identified in the photographs of the autoradiogram due to the close proximity of the host protein and their failure to react with the various human sera tested in the immunoprecipitation reaction. Examples of three such proteins are proteins with molecular weights around 120, 102, and 49. The differential response of various proteins can again be seen in Fig. 1D, confirming the data presented in Fig. 1A.

Proteins 138, 80, and 45 are of particular interest because they are relatively insensitive to canavanine treatment and because cells containing these proteins are positive for EA. We (1a) and others (2, 5, 6) have shown that proteins with the same or similar molecular weights are specifically precipitated from extracts of EA-positive cells by EA^+ VCA^+ sera but not by EA^- VCA^+ sera, suggesting that indeed EA synthesis can be blocked by canavanine when the correct conditions are chosen.

The different effects of canavanine on the various groups of proteins could be explained as follows. A group of proteins (primary proteins; 1a) are synthesized which exert a controlling function: when they are made in an active form, the cells can then synthesize proteins belonging to the secondary group, whereas in the presence of 2.7 mM canavanine and the absence of arginine, they are made in an inactive form and cannot initiate secondary protein synthesis. If, however, enough arginine is present in the medium a percentage of the primary proteins will be synthesized in an active form and can initiate the synthesis of secondary proteins (such as proteins 138, 80, and 45); if even more arginine or less canavanine is present, then a certain percentage of the secondary proteins will be made in an active form and can initiate the synthesis of the third group of proteins (e.g., 143).

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