The appearance of ^{3}H-thymidine incorporating large lymphoblasts has been observed in nodes draining the graft bed before graft rejection in animals[1]. The appearance of these cells heralds the development of immunological commitment to the graft antigens. During effective immunosuppression the development of committed effector lymphocytes from these cells is inhibited[2]. During the immune response to antigenic stimuli, including grafts, large numbers of these large lymphoid cells are produced by proliferation, are released into the efferent lymph, enter the blood and ultimately invade the graft bed where they act as effectors of cellular immunity[3]. This phenomenon also occurs during the response of man to typhoid vaccine and the cells can be detected in the peripheral blood by morphological examination or by measurement of thymidine incorporation[4].

On the basis of these findings, we postulated that the activated lymphocytes would appear in the circulation and could be detected during or even preceding clinical evidence of graft rejection. The experimental data support this hypothesis and suggest that the serial measurement of peripheral blood activated lymphocytes will provide data which can predict by 24–48 h an episode of organ allograft rejection.

This study was supported by a contract from the Collaborative Research Program, Transplantation Immunology Branch, US National Institutes of Allergy and Infectious Disease, and grants from the US National Institutes of Health to the General Clinical Research Center and the Cardiovascular Research and Training Center of Baylor Medical College.

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Received January 30; revised March 2, 1970.

[1] Andre, J., Schwartz, R., Mitus, W. J., and Damashek, W., *Blood*, **19**, 313 (1962).
[2] Andre, J., Schwartz, R., Mitus, W. J., and Damashek, W., *Blood*, **19**, 334 (1962).
[3] Hall, J. G., *J. Exp. Med.*, **125**, 737 (1967).
[4] Crowther, D., Fairley, G. H., and Sewell, R. L., *J. Exp. Med.*, **129**, 849 (1969).

Blood Group H Antigen of Influenza A2 Virus

McDonald and Zuckerman[1] have demonstrated statistically that there are a large number of blood group O patients and a relatively few of group A among those infected with influenza A2 virus. Subsequent studies[2–4] have indicated, however, that an antibody against influenza was more commonly found in sera of group O persons than group A. Potter and Schild[2] suggested that such a divergency in influenza infection was influenced by genetic factors related to ABO blood groups. We have examined influenza viruses for blood group antigens in order to obtain evidence for any possible relationship between influenza and ABO blood groups.

We used allantoic fluid from hens' eggs inoculated with influenza A, A2 or B virus. The fluid was centrifuged at low speed to remove membrane debris and centrifuged for a further 3 h at 100,000*g* to sediment viruses; the sediment was washed by resuspending it in buffered saline (*p*H 7·0) and then centrifuging. The final sediment was suspended homogeneously in buffered saline, and its agglutinating titre with chicken red cells adjusted to 1 : 2,048 by dilution. The suspension was heated for 20 min in boiling water. Preliminary tests of agglutinin inhibition showed no decrease in titre of anti-A, anti-B and anti-H agglutinins. The suspension was then examined by an absorption–elution test in the following way. Thin pieces of paper (3 × 5 mm) were dipped into a serially diluted row of the suspension, containing 2·5 per cent hen's egg albumin, and dried at room temperature. The papers were immersed in acidified methanol for 20 min and dried for 4 h at 56° C; they were then placed in 1 ml. of potent anti-H reagent (extract of *Ulex europaeus* seed) and allowed to stand overnight at 2° C, after which time they were washed four times in a sufficient volume of normal saline. After the addition of normal saline (0·09 ml.) the papers were heated at 50° C for 20 min in a water bath. Eluted agglutinin in the normal saline was separated and a serial dilution was made immediately.

Table 1. COMPARISON OF RESULTS OF ANTI-H ABSORPTION–ELUTION TESTS OF INFLUENZA A, A2 AND B VIRUSES

Virus	Titres of anti-H obtained by tests of undiluted samples	Minimum virus* concentrations to detect anti-H
Influenza A	< 1	> 2,048
Influenza A2 (Kumamoto)	8	64
Influenza A2 (Hongkong)	8	128
Influenza B	1	2,048
Control	< 1	—

* Represented by titres of chicken red cell agglutination.

Agglutinin tests of the eluate were performed by the immediate addition of a 1/2 part of 0·5 per cent red cell suspension (group O) to the diluted row and by low speed centrifugation after addition of 1/2 part of agglutinin-free human group AB serum following 15 min storage at 5° C. Tests of papers adsorbing the undiluted suspension of influenza A2 viruses gave relatively high titres of anti-H activity (Table 1); the influenza A virus proved to have a trace of anti-H in an eluate; the B virus gave a weakly positive reaction. The minimum concentrations of the viruses required for the detection of anti-H were low for A2 viruses and high for A and B. A control experiment was simultaneously performed using paper adsorbing non-inoculated egg allantoic fluid plus 2·5 per cent egg albumin, but the results of absorption–elution were negative. Detection of blood group A and B antigens was also performed, but the results obtained were not reliable, because in some experiments anti-A and anti-B were eluted from papers with fixed egg albumin only.

Our results establish the presence of blood group H antigen in influenza A2 and B viruses, the quantity being more in A2 than in B. We conclude that anti-H agglutinin is involved in the prevention of certain influenza infections. The relatively high incidence of group O patients infected with some types of influenza may be attributable to the lack of anti-H antibody in their sera or secretions.

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Received October 30, 1969; rsevied January 19, 1970.

[1] McDonald, J. C., and Zuckerman, A. J., *Brit. Med. J.*, II, 89 (1962).
[2] Potter, C. W., and Schild, G. C., *J. Immunol.*, **98**, 1320 (1967).
[3] Shedden, W. I. J., and Potter, C. W., *Nature*, **202**, 505 (1964).
[4] Tyrrell, D. A. J., Sparrow, P., and Beare, A. S., *Nature*, **220**, 819 (1968).