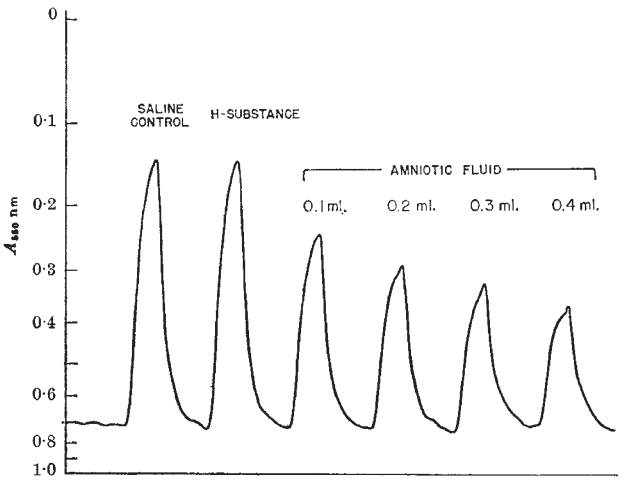

Fig. 1. Result of addition of saline, purified H substance, and increasing amounts of amniotic fluid to an anti-I cold agglutinin before quantitation of the agglutination. The purified H substance failed to cause any inhibition of the agglutination, but the amniotic fluid caused strong inhibition which was proportional to the amount of amniotic fluid used.

patients with infectious mononucleosis[6]. Very large amounts of cold agglutinin, usually of the anti-I specificity, are made by rare adults with the chronic cold haemagglutinin disease[7]. Studies of the antibodies produced by these patients have demonstrated that they are monoclonal[8–10].

A recent abstract[11] describing the presence of soluble I substance in human milk prompted work which has revealed the presence of soluble I substance in human amniotic fluid and urine, and confirmed its presence in milk.

Agglutination of normal adult I red cells by anti-I cold agglutinins, and the inhibition of this agglutination, were measured by a sensitive and reproducible method involving the Technicon autoanalyser. Aliquots of anti-I were sampled and mixed with a continuous flow of 20 per cent red cells. Agglutinated red cells were subsequently removed at T-junctions, and the remaining unagglutinated cells lysed and their haemoglobin quantitated from $A_{550\ nm}$. The baseline of the recording represented absence of agglutination and had the highest $A_{550\ nm}$. Anti-I activity resulted in peaks with a diminished $A_{550\ nm}$. An inhibitory substance added to the anti-I solution caused diminution in the height of the peak.

For most of the inhibition studies, a single potent anti-I cold agglutinin from a patient (J. R.) with the chronic cold haemagglutinin disease was used at a titre of 1/67,000. But similar results were obtained with other high titre anti-I antibodies, containing normal anti-I sera, and with transiently elevated anti-I antibodies.

Many samples of human amniotic fluid, obtained at the time of delivery or from amniocentesis between the twenty-first and thirty-fourth weeks of gestation, were all found partially to inhibit agglutination by the anti-I. Increasing the amount of amniotic fluid caused increasing inhibition of the agglutination. By contrast, purified H substance (from Dr W. M. Watkins) tested at a concentration of 0·8 mg/ml. did not inhibit anti-I (Fig. 1). The inhibition of the anti-I by the amniotic fluid occurred very rapidly and was as effective at 37° C as at 4° C. The inhibitory effect was not altered by boiling the amniotic fluid for 5 min but was prevented by precipitation with trichloroacetic acid of proteins or by treatment of the amniotic fluid with 0·01 M periodate at 37° C for 4 h. The inhibitory substance was non-dialysable and was estimated to have a molecular weight of approximately 10^6 because of its exclusion from 'Sephadex G–200' and near exclusion from 'Biogel A–5'.

The presence of anti-I inhibiting substance was confirmed in human milk[11], and this substance had the same properties as that in amniotic fluid. Urine from adults, pregnant women, and newborn babies contained some inhibitory substance, though usually not as potent as that in amniotic fluid and milk. Follow-up urine samples from two 3 week old infants were negative although they had strong inhibitory activity during the first 24 h of life. The inhibitory substance in urine was largely dialysable and was retained on 'Sephadex G–200', appearing with the maximum colour. Saliva contained minimal inhibitory substance and cerebrospinal fluid had no activity.

The inhibitory substances found in amniotic fluid, milk and urine are believed to be specific soluble I antigen although it is not known whether these substances bind to the anti-I; nor whether they are derived from the red cell membrane or are produced by secretory cells. The I substance in amniotic fluid may be of maternal origin and it—or a low molecular weight derivative—may enter the foetus and stimulate the production of the anti-I found at birth. Purification and characterization of the I substance from amniotic fluid are in progress.

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Antibody and Complement-like Factors in the Cytotoxic Action of Immune Lymphocytes

L5178Y lymphoma cells from DBA/2 mice grow well *in vitro* and the cytotoxicity of immune lymphoid cells can be assayed easily in terms of the degree to which they inhibit the growth of the lymphoma. Using this system we have already shown that sheep lymph cells, collected from the efferent ducts of individual lymph nodes that had been immunized with the lymphoma, displayed a powerful and immunologically specific cytotoxic action[1]. The cytotoxicity of the lymphoid cell population correlated with the percentage of large basophilic immunoblasts that were present and consequently an unequivocal cytotoxic action was only demonstrable between 4–7 days after immunization when large numbers of immunoblasts were present in the lymph. At later times the lymph was populated mainly with small lymphocytes and these cells, even when collected from hyperimmune sheep, were without demonstrable cytotoxic action *in vitro*. For these reasons it was concluded that the immuno-

blast was the cytotoxic cell. In these experiments the lymphoma cells were cultured in a medium containing 10 per cent heat-inactivated foetal calf serum.

It was assumed originally that serum complement factors played no part in the cytotoxic action, although later experiments showed that the cytotoxicity of sheep lymph cells was always increased if non-inactivated foetal calf serum was included in the cultures. This foetal serum had no complement activity in terms of its ability to lyse antibody-coated sheep red cells but nevertheless was able to lyse lymphoma cells that had been coated with allo-antibody from inactivated serum of C57/Bl mice hyper-immunized with L5178Y lymphoma cells. These findings drew our attention to the possible importance of complement in cytotoxic reactions and led us to investigate in detail the effects of adding fresh sera with high complement activity. Guinea-pig serum could not be used because of its asparaginase activity and rabbit sera were often directly toxic to the lymphoma. Thus we used fresh serum from specific pathogen free Wistar rats which had neither of these disadvantages and yet contained adequate complement activity. The results (Table 1) indicate that in certain conditions the cytotoxic activity of the sheep lymphoid cells was increased up to forty-fold by the presence of fresh sera, whereas the addition of heat-inactivated sera had no such effect. The addition of fresh serum was unnecessary for the cytotoxic reaction when immune lymphocytes were added at a ratio of 100 or more for each target lymphoma cell. This suggests that lymphoid cells either contain or synthesize some components of the complement system. Complementary activity of lymphoid cells has been established by others[2–4], and we have obtained evidence that this complement-like activity is associated with the immunoblasts rather than with the small lymphocytes.

Table 1. THE EFFECT OF THE ADDITION OF FRESH SERUM ON THE INHIBITION OF GROWTH OF L5178Y LYMPHOMA BY LYMPH CELLS COLLECTED FROM THE LYMPH OF SHEEP 4 TO 7 DAYS AFTER IMMUNIZATION WITH LYMPHOMA

Ratio of sheep lymph cells to lymphoma cells	Percentage growth inhibition after 48 h culture in Fischer's medium containing: 10 per cent FCS inactivated	10 per cent normal FCS	10 per cent inactivated FCS + 3 per cent inactivated rat serum	10 per cent inactivated FCS + 3 per cent normal rat serum
0 : 1 (control)	9	0	0	0
5 : 1	2	15	2	90
25 : 1	0	75	5	100
100 : 1	19	100	11	100
200 : 1	91	100	89	100

FCS stands for foetal calf serum.

The increase in the cytotoxic effect with complement leads us to conclude that specific antibody must be involved in the cytotoxic event, and it seems clear that the immunoblasts must be the source of this antibody[5,6]. This proposition was tested by showing that the complement-dependent cytotoxic reaction could be inhibited by the addition of rabbit anti-sheep γ-globulin serum. In these experiments the cytotoxicity of the sheep lymphoid cells was measured in terms of the release of radioactivity from target lymphoma cells that had been labelled with ^{51}Cr (ref. 7); the results are shown in Table 2. The presence of anti-sheep γ-globulin serum totally abolished the cytotoxic effect of the lymphoid cells, yet at the concentration at which it was used this antiserum did not impair the structural integrity or motility of the sheep immunoblasts.

These results strongly suggest that the destruction of xenogeneic lymphoid target cells by specifically sensitized sheep immunoblasts can be brought about by the antibody and complement that are associated with the immunoblasts. Most investigators have found that the optimum expression of this cytotoxic activity in "complement-free" systems usually depends on the presence of large numbers of viable immune lymphoid cells and their close contact with the target cells[8–10]. We have found, however, that when enough serum complement is added to the system,

Table 2. INHIBITION OF CYTOTOXIC ACTIVITY OF IMMUNE SHEEP LYMPH CELLS BY RABBIT ANTI-SHEEP-γ-GLOBULIN SERUM

Treatment of lymphoid cells	Percentage ^{51}Cr release (±s.e.) from L5178Y lymphoma cells in the presence of: 3 per cent inactivated rat serum at: 2·5 h	8 h	3 per cent fresh rat serum at: 2·5 h	8 h
No lymphocytes added	26±1	41±1	27±2	42±2
Immune lymphoid cells added to lymphoma at ratio of 100 : 1	25±5	54±1	84±8	96±1
Immune lymphoid cells added at above ratio together with anti-sheep γ-globulin serum*	25±3	39±2	23±1	41±3

* Rabbit anti-sheep-γ-globulin serum inactivated at 56° C for 45 min, absorbed for 1 h at 4° C on equal volume of washed lymphoma cells; used at a final concentration of 3 per cent.

immunoblasts retain their specific ability to inhibit the growth of lymphoma cells even though they are separated from them by a 'Millipore' membrane of 0·45 μm pore diameter. In other words, some of the lytic antibody is released into the culture medium but the complement-like activity can only be expressed when there is close contact between the immune and the target cells. Extrapolation of these *in vitro* studies to rejection of grafts *in vivo* must take into account the coexistence of many ancillary mechanisms that may be crucial in that situation.

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Localization of Afferent Lymph Cells within the Draining Node during a Primary Immune Response

LYMPH nodes can receive cells from the blood and from the lymph. The principal pathway from the blood into the nodes is through the post capillary venules in the paracortex[1], the second is through the afferent lymphatics. The number of cells in the afferent lymph increases after the injection of an antigen into the drainage area[2], but their localization in the node and their role in mediating the immune response are unknown. Obstruction of the lymphatics reduces the size and number of germinal centres within the node[3], suggesting a relationship between these structures and the afferent lymph. The work reported here was concerned with the kinetics of the cellular response in the afferent lymph after antigenic stimulation and the fate of these cells within the node.

New Zealand white rabbits were inoculated intradermally in three sites on the ventral aspect of each hind foot with either 10 Lf of PTAP (10 guinea-pig lethal