Lysis of Human Cultured Lymphoblastoid Cells by Cell-Induced Activation of the Properdin Pathway

Abstract. *Human cultured lymphoblastoid cells incubated in normal human serum activate the properdin complement pathway without antibody. However, only cells bearing C3b immune adherence receptors bind components of the properdin complement pathway and undergo lysis. A similar surveillance mechanism may exist in man to limit growth of malignant B cells bearing C3b immune adherence receptors.*

Activation and subsequent fixation of complement on cells may irreversibly alter the cell membrane or may activate specialized cell functions. Accordingly, complement has been defined as an extracellular effector and modifier of biological membranes. The complement system, as reviewed by Müller-Eberhard (*1*), has been subdivided into two pathways of activation. The first or classical pathway is activated by immune complexes of the immunoglobulin (Ig)M or IgG type, which interact with complement component C1 (recognition unit). Thereafter, the activation unit (C4, C2, and C3) assembles, followed by the membrane attack unit (C5 to C9). The second or properdin (alternative) pathway is activated by aggregates of IgA, polysaccharides, lipopolysaccharides, or cell-bound IgG. It consists of at least five proteins, the initiating factor, properdin, C3, factor B, and factor D. The properdin pathway of complement activation bypasses C1, C2, and C4 and at the C3 stage meets the classical pathway. Bone marrow–derived (B-type) lymphocytes may possess two types of receptors for fragments of C3: one for C3b (immune adherence receptor) and one for C3d (*2*). An additional binding site on C3b for cell membranes is short-lived and allows attachment of C3b to a receptor site on the cell other than the immune adherence receptor site. The biological function of the receptors for the stable binding sites on C3b has not yet been defined, but several hypotheses have been proposed [reviewed in (*3*)].

We recently showed that incubation of human cultured lymphoblastoid (HCL) cells with normal human serum (NHS) results in activation of the properdin pathway (*4*). However, after activation only cells bearing C3b immune adherence receptors were found to bind C3b, properdin, and factor B. No other serum factors were needed to mediate the binding of C3 or C3b to cellular complement receptors. However, binding of properdin and factor B required the presence of C3b, and stabilization of cell-bound factor B necessitated the presence of activated properdin (*4*). We report here lysis of HCL cells bearing C3b immune adherence receptors by NHS, mediated by cell-induced activation of the properdin pathway.

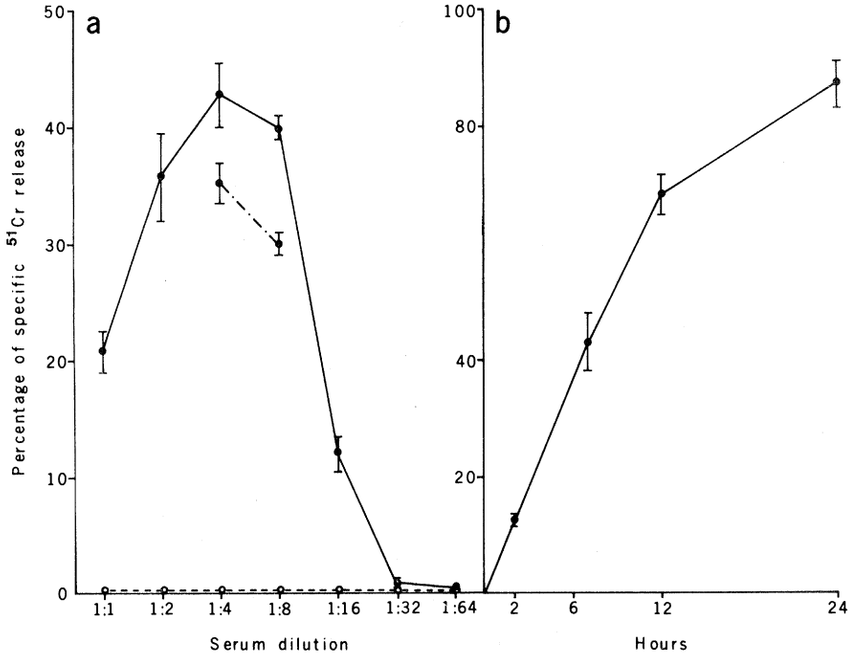

Fig. 1. (a) Lysis of RAJI cells by NHS. RAJI cells (2×10^7) were labeled with 100 μc of ^{51}Cr ($Na_2{}^{51}CrO_4$, New England Nuclear). In triplicate, 1×10^5 labeled RAJI cells in 200 μl of solution A [Veronal-buffered saline (VBS) with Ca^{2+}, Mg^{2+}, and 10 percent fetal calf serum inactivated by heating at 56°C for 45 minutes] were incubated (7 hours at 37°C) in flat-bottom microtiter plates with 100 μl of fresh NHS serially diluted in solution A (•—•), absorbed twice at 4°C for 30 minutes with 2×10^7 RAJI cells (• – · – · – •), or heated at 56°C for 45 minutes (○ - - - ○). After centrifugation of the plates, 200 μl of the supernatant was removed and counted. Percentage of specific ^{51}Cr release was calculated by the formula: 100 × (experimental − spontaneous)/(maximal − spontaneous). Spontaneous release was determined by incubating cells in solution A and maximal release by incubating cells in 0.5 percent nonident P40. (b) Time course of RAJI cell lysis by fresh NHS. The test was performed as above by using a 1 : 4 dilution of NHS. Spontaneous ^{51}Cr release was determined by incubating cells for the indicated time intervals in NHS heated at 56°C for 45 minutes. The NHS used in all the experiments was obtained from one healthy subject and used fresh or after storage at −70°C. Normal human serums from five other healthy individuals tested for lysis of RAJI cells were equally active. Each point represents the mean ± standard deviation (S.D.) of five independent experiments.

Lysis of RAJI cells, a human B-type cell line derived from a Burkitt's lymphoma (*5*), by fresh NHS was demonstrated by ^{51}Cr release (Fig. 1a). Optimum lysis was observed with 1 : 4 and 1 : 8 dilutions of fresh NHS. The presence of an inhibitor, possibly C3b inactivator, may account for the greater lysis observed with the diluted serum than with the neat serum. The C3b inactivator may impair the function of C3b in the feedback mechanism of the properdin pathway (*6*), may act directly on factor D, leading to inhibition of this enzyme (*7*), or may destroy the receptor site for properdin on C3b (*8*). Prior absorption of the serum with RAJI cells at 4°C had little effect on its lytic activity. However, heating of NHS at 56°C for 45 minutes abolished the lytic activity, indicating that lysis was mediated by complement.

Figure 1b depicts the time course of the lytic reaction; significant isotope release occurred within 2 to 3 hours and the reaction progressed to near completion by 24 hours. In subsequent experiments a 7-hour incubation period was used. The complement pathway responsible for the lysis of RAJI cells was sought in the following experiments, in which ^{51}Cr-labeled cells were incubated with serums as described.

1) Normal human serum first heated at 50°C for 25 minutes to destroy serum factor B (*9*). Lysis was not observed.

2) A 1 : 4 dilution of either NHS or human serum congenitally deficient in C4 or C2 and free of immune complexes (*10*). In three experiments, human se-

rums deficient in components that are essential in activating the classical complement pathway produced specific ^{51}Cr release (45.8 ± 3.4 and 40.0 ± 4.2 percent, respectively) identical to that produced by NHS (43 ± 5.5 percent).

3) A 1:4 dilution of NHS containing 10 m*M* ethylenediaminetetraacetic acid (EDTA, blocks both complement pathways) (*11*), 10 m*M* [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA, blocks the classical complement pathway) (*11*), or 10 m*M* EGTA plus 1.5 m*M* Mg^{2+}. Spontaneous isotope release was determined by incubating cells in NHS that had been heated at 56°C for 45 minutes and to which 10 m*M* EDTA or EGTA had been added. In four experiments, EGTA did not affect lysis of RAJI cells by NHS (42.0 ± 3.9 percent specific ^{51}Cr release in EGTA-NHS, 46.8 ± 5.1 percent in EGTA-Mg^{2+}-NHS, and 44.5 ± 2.5 percent in NHS). However, EDTA abolished the lytic effect of NHS on RAJI cells.

4) Normal human serum depleted of C4 or factor B (Fig. 2a). Depletion of C4 had relatively little effect on lysis, but depletion of factor B abolished the lytic activity.

5) Normal human serum previously heated at 50°C for 25 minutes and then reconstituted with increasing amounts of purified human factor B. The lytic activity of the heated serum increased with addition of factor B (Fig. 2b), such that replacement of factor B to physiologic levels almost completely restored the lytic activity.

These experiments establish that lysis of RAJI cells by fresh NHS is primarily mediated by activation of the properdin pathway.

Other HCL cells of bone marrow origin (Daudi, Wil 2WT, and 8866) and thymus (T) origin (MOLT-4), which we have shown to activate the properdin pathway after interaction with NHS, were also tested for lysis. Substantial lysis (25.6 ± 2.3 percent specific ^{51}Cr release at 7 hours) occurred with Daudi cells that have C3b immune adherence receptors (*12*). However, with the other HCL cells, which did not have C3b immune adherence receptors (*12*), lysis did not occur. Human peripheral lymphocytes (HPL's) obtained from five healthy donors were also tested for activation of the properdin pathway and lysis. They were used immediately after isolation or after culturing for 4 days in the same medium as that used for the HCL cells (*4*). Immunoelectrophoretic and immunofluorescence studies showed neither activation nor fixation of C3, properdin, or factor B to these cells. Accordingly, ^{51}Cr-labeled normal HPL's and isolated T or B cells (*12*) that interacted with fresh NHS were not lysed. Furthermore, isolated C receptor–bearing normal HPL's, first stimulated to undergo blast transformation between 50 and 60 percent blasts at 4 days) with staphylococcal lysate (*13*), failed to lyse in NHS.

The results reported here, in conjunction with our previous findings (*4*), indicate that HCL cells, in contrast to normal or mitogen-stimulated HPL's, activate the properdin pathway and are lysed. The activation of the properdin pathway by lymphoblastoid cells has been confirmed by Budzko *et al.* (*14*). The initial event appears to occur on the surface of the lymphoblastoid cells, since supernatants obtained from the cultured cells were unable to trigger properdin pathway activation. It is assumed that after interaction of the lymphoblastoid cell membrane structure with a principle in serum, possibly the initiating factor (*8*), activation of the properdin pathway ensues with deposition of C3b–properdin–factor B complexes (*4*) onto the cellular C3b immune adherence receptors. Thereafter, cell-bound C3-C5 convertase (*8, 15*), which is stabilized by properdin and dependent on factor B, initiates utilization of the terminal components of complement, resulting in the assembly of the membrane attack unit and cell lysis. In previous experiments (*16*) with different culture conditions, the inability to detect significant lysis of RAJI cells cultured in NHS was probably related to the absence or presence in insufficient amounts of a serum component, such as properdin, necessary for the cell-induced activation of complement. To activate the properdin pathway, HCL cells may possess membrane-associated activating enzymes or factors. Alternatively, the surface membranes of these cells may carry virus or viral proteins capable of activating complement in the absence of antibody (*17*). Finally, activation of the properdin pathway may result from immune complex formation between antibodies in serum and antigenic cell membrane constituents. Cells that were infected with a variety of RNA and DNA viruses and then interacted with specific immune fresh human serums were apparently lysed by activation of the properdin pathway (*18*). In our system antibodies reacting against antigens on the surface of HCL cells do not appear to participate in the activation of the properdin pathway by HCL cells.

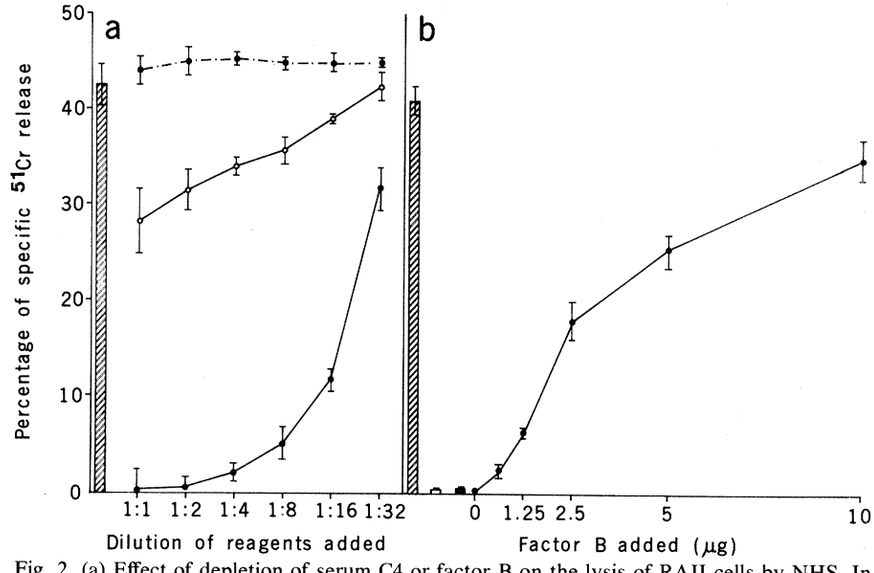

Fig. 2. (a) Effect of depletion of serum C4 or factor B on the lysis of RAJI cells by NHS. In triplicate, 1 × 10^5 ^{51}Cr-labeled RAJI cells in 100 μl of solution A (see Fig. 1) were incubated (7 hours at 37°C) with 100 μl of a 1 : 4 dilution of fresh NHS, which was first incubated (4°C for 15 minutes) with 100 μl of a serial dilution of the Fab′ portion of goat antibody to human C4 (○——○), human factor B (●——●), or (as a control) human IgG (● – · – · – ●). Addition of the first two of these resulted in complete depletion of C4 or factor B, respectively. The degree of C4 or factor B depletion was determined (*18*). Purified antiserums and their Fab′ fragments were prepared as described in (*18*). Lysis observed with untreated fresh NHS is also illustrated (hatching). (b) Restoration of the cytolytic activity of human serum heated at 50°C for 25 minutes by the addition of purified factor B. In triplicate, 1 × 10^5 ^{51}Cr-labeled RAJI cells in 150 μl of solution A were incubated with 100 μl of a 1 : 4 dilution of human serum which had been heated at 50°C for 25 minutes and then reconstituted with 50 μl of a serial dilution of purified human factor B (●——●). Factor B was purified as described in (*4*) and used at a concentration of 200 μg per milliliter of VBS with Ca^{2+} and Mg^{2+}. Cells were also incubated with NHS (hatching), human serum heated at 50°C for 25 minutes but not reconstituted with factor B (□), or 20 μg of purified factor B alone (■). Each point represents the mean ± S.D. of three independent experiments.

First, antiserums labeled with fluorescein failed to detect immunoglobulins on the surfaces of MOLT-4 cells pretreated with NHS. These cells were tested because they lack membrane-bound immunoglobulin and fragment c receptors (*19*). Second, incubation of RAJI cells with NHS heated at 56°C for 45 minutes failed to induce lysis of these cells after addition of fresh guinea pig serum as a source of complement. The guinea pig serum supported complement-dependent lysis of erythrocyte-antibody complex but was minimally lytic (2.5 ± 0.9 percent ^{51}Cr release) for RAJI cells. Third, as indicated above, absorption of fresh NHS with RAJI cells at 4°C had little effect on its lytic activity against these cells. Fourth, serums from two agammaglobulinemic patients lysed RAJI cells as efficiently as NHS. In addition, lysis of RAJI cells is probably not produced by antibodies to Epstein-Barr virus (EBV), since these cells, although carrying a repressed EBV genome, are free of detectable EBV-related antigens (*20*).

Activation of the properdin pathway and lysis of lymphoblasts bearing C3b immune adherence receptors may represent a natural mechanism of in vivo surveillance to limit the growth of B-type lymphoma and lymphoblastic leukemia cells. The antitumor effect of certain polysaccharides is related directly to their capacity to activate the properdin pathway (*21*), while infusion of fresh, but not heated, NHS has been used to treat leukemia in AKR mice (*22*). It is possible that an absolute or functional deficiency of a properdin complement pathway component may exist in patients with leukemia or lymphoma, which would limit the efficiency of lysis of tumor cells bearing C3b immune adherence receptors mediated by this pathway. It would be worth knowing whether B-type lymphoma and leukemia cells obtained directly from patients have components of the properdin system fixed to their surface membranes and whether the properdin pathway is activated in their serums.

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Angiogenesis: A Marker for Neoplastic Transformation of Mammary Papillary Hyperplasia

Abstract. *Mouse mammary papillomas elicit new formation of vessels when transplanted onto the rabbit iris. This angiogenic capacity is a property of carcinomas but not of the resting mammary gland. In mouse papillary hyperplasias, however, this property appears much earlier than any morphological or clinical sign of carcinoma. A test for angiogenic capacity may reveal a step in the progression toward clinical malignancy and thus could be used to screen for neoplastic potential of hyperplastic epithelium in biopsy tissues.*

The hypothesis that overt clinical neoplasia develops in cell populations through a sequence of changes that are often disguised as hyperplasia has been extensively debated and is accepted by many cancerologists (*1–3*). The mammary gland is an organ particularly prone to hyperplastic lesions. Some of them have been labeled preneoplastic to suggest their high risk of becoming clinically

Table 1. Angiogenic response of benign mouse mammary papillomas.

Experiment	Transplant generation	Latent period (weeks)	Fraction of iris implants with neovascular response		
			Tumor	Boiled tumor	Mouse liver
		C57BL mice (urethane-induced)			
1	2	14	19/19	0/3	
2	2	14	23/23	0/7	
3	1	15	32/32	0/5	0/5
4	1	15	13/13		0/9
5	3	14	19/19	0/12	
6	3	14	30/30	0/7	
7	3	14	29/29	0/9	
8	1	47	34/34	0/11	
9	1	51	25/26	0/6	
		BALB/c mice (DMBA-induced)			
10	3	13	26/26	0/16	
11	5	10	23/24		0/10
12	5	10	18/18		0/16
13	1	16	16/16		0/17
Total			307/309	0/76	0/57
Percentage			99	0	0