between Moose Factory and Fort Albany (*7*).

The content analysis procedures developed to objectively extract the dates described above are fully elaborated elsewhere (*8*) and can, with modification, be employed in any environmental research into descriptive, historical accounts. Thus, the method can be extended to a variety of environmental phenomena and to periods and regions for which instrumental data are lacking.

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Expression of C4 on Human Lymphoid Cells and Possible Involvement in Immune Recognition Phenomena

Abstract. *Immunochemical studies revealed the presence of the fourth component of complement (C4) on surfaces of human lymphoid cells. Antiserums to C4 inhibited the mixed lymphocyte reaction and the mitogenic response to phytohemagglutinin, suggesting a role for membrane-associated C4 in the afferent phase of immune recognition phenomena.*

Genes located in the major histocompatibility complex (MHC) specify antigens and control functions concerned with immune recognition. In man, the genes coding for antigens involved in allograft rejection reside in three regions termed locus A, locus B, and locus D (formerly denoted as the LA, FOUR, and MLR regions, respectively). Antigens determined by locus D regulate allogeneic recognition in the mixed lymphocyte reaction (MLR) and are now characterized by quantitating the incorporation of [^{3}H]thymidine in mixtures of allogeneic lymphocytes after 5 days of culture at 37°C. The products of the locus A and locus B regions of the HLA system are target antigens for cell mediated lymphocytotoxic reactions (*1*), and are readily detected on cells and in serum with monospecific antiserums in a cytotoxic test dependent on complement (C). Genes regulating the lytic activity of serum complement in mice (*2*) and the expression of several complement components, including the third component (C3) in mice (*3*), factor B in monkeys (*4*), and the second component (C2) (*5*) and factor B (*6*) in man, are linked to genes governing the expression of MHC antigens. These observations led to our investigation of whether complement components might be present on the cell surface, as are other MHC coded antigens, and whether they play a role in cellular immune recognition phenomena. We report here that the fourth component (C4) is expressed on the surfaces of human lymphoid cells and that antiserums to C4 interfere with allogeneic recognition in the MLR.

Human lymphoid cell lines Raji, RPMI 1301, RPMI 1788, and WI-L2 were grown in suspension cultures in RPMI 1640 medium supplemented with 10 percent fetal calf serum (*7*). Exponentially growing cells were used for most studies.

Human peripheral lymphocytes were isolated from heparinized blood by centrifugation on Ficoll-Hypaque after plasmagel sedimentation of erythrocytes (*8*). The cells were washed with Hanks balanced salt solution (BSS) before use.

Antiserums to isolated human C4 (*9*) were raised in rabbits and goats. Antiserums were also produced in rabbits to human C4 attached to autologous rabbit erythrocytes by the action of activated C1s (*10*).

Operationally monospecific HLA alloantiserums were obtained from the serum bank at the National Institute of Allergy and Infectious Diseases.

The four goat antiserums and four of the five rabbit antiserums used in these studies were monospecific for C4 without absorption, while the fifth rabbit antiserum was monospecific after absorption with isolated immunoglobulin G (IgG).

We used two techniques to demonstrate C4 on the lymphoid cell surfaces. First, varying numbers of washed, packed, cultured lymphoid cells were incubated with undiluted rabbit or goat antiserums to C4 (anti-C4). Depletion of anti-C4 reactivity was assessed by reduced ability of the antiserum to precipitate C4 after immunoelectrophoretic separation in agar. Cultured human lymphoid cells RPMI 1788 produced a dose-dependent absorption of rabbit antibody to C4 with the largest number of cells used (1.2×10^7 per microliter) leading to virtually complete absorption. Similar results were obtained with WI-L2 cells and with two other rabbit antiserums and one goat antiserum to C4. Since the absorption procedure produced only a twofold dilution of the antiserum as evidenced by dilution of ^{125}I-labeled human albumin incorporated in the antiserum, the absorption of anti-C4 reactivity cannot be explained by dilution of the antibody.

Second, we quantitatively determined the expression of C4 on surfaces of cultured human lymphoid cells by a slight modification of the method of Ohanian and Borsos (*11*). Varying numbers of packed, washed lymphoid cells, typically 3×10^8, 1.5×10^8, 7.5×10^7, and 3.7×10^7, were incubated with 150 μl of an appropriate concentration of the IgG fraction of antiserum to C4. After 90 minutes at 37°C, the lymphoid cells were removed by centrifugation, and 100 μl of the supernatant was assayed for remaining anti-C4 reactivity by measuring its ability to sensitize sheep erythrocytes coated with C4 (EC4) (*10*), for lysis by guinea pig complement. The concentration of the IgG fraction of the anti-C4 used in our studies produced 90 percent lysis of EC4. A reduced amount of lysis was obtained after incubation of the antiserum with a source of C4. An inhibition curve with isolated C4 was included in all experiments that permitted conversion of the amount of lysis observed after incubation of the antiserum with lymphoid cells into nanograms of C4. These values were then converted to C4 molecules per lymphoid cells; we used a molecular weight of 204,000 for C4 (*9*). The numbers of C4 molecules per cell were 487 for Raji cells, 1150 for RPMI 1301 cells, 700 for RPMI 1788 cells, and 680 for WI-L2 cells. Two different antiserums to C4 and two C4 preparations produced comparable results. We used two controls. First, 5×10^9 human erythrocytes and sheep red blood cells were used to absorb the anti-C4. No absorption could be detected. Second, since cultured human lymphoid cells were perpetuated in medium containing 10 percent fetal calf serum, the ability of fetal calf

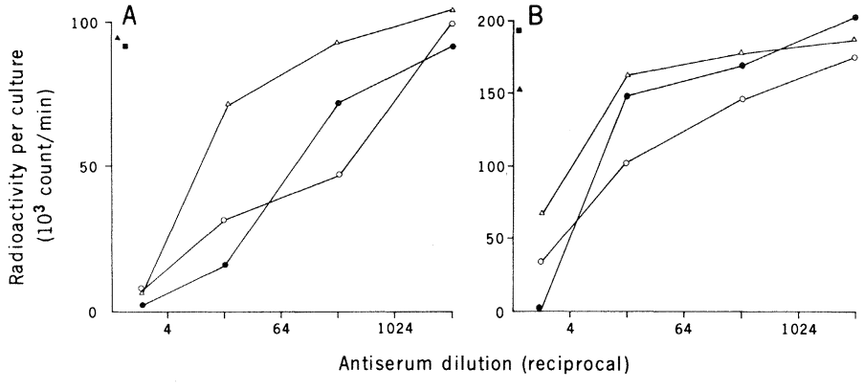

Fig. 1. Inhibition by antiserums to C4 of the unidirectional mixed lymphocyte reaction between allogeneic human peripheral lymphocytes (A) and of the mitogenic activity of PHA for human peripheral lymphocytes (B). (A) The mixed lymphocyte reaction was performed (*19*) with 1×10^5 peripheral lymphocytes as responding cells and 1×10^5 irradiated (3000 roentgens) peripheral lymphocytes or 2.5×10^4 irradiated cultured lymphoid cells as stimulator cells. [^{3}H]Thymidine (2 μc) (New England Nuclear, Los Angeles) was added per culture (250 μl final volume) after 5 days of incubation at 37°C. Cultures were harvested after an additional 12 to 16 hours incubation, and incorporation of [^{3}H]thymidine was measured by counting the samples in a Packard liquid scintillation spectrometer. In our test the variation coefficient from triplicate experiments is less than 15 percent. Results with two rabbit antiserums (△;○) and one goat antiserum (●) are shown. Control cultures contained normal rabbit serum (▲) or normal goat serum (■). (B) The effect of antibody to C4 on the mitogenic activity of PHA was tested by incubating 2×10^5 peripheral lymphocytes with 0.12 μg of PHA M (Difco, Detroit) for 4 days at 37°C at which time [^{3}H]thymidine was added. After an additional 12 to 16 hours of incubation, incorporation or radioactivity was determined. Symbols are as described in (A).

serum to interact with rabbit antiserum to human C4 was tested. No inhibition of the cytolytic potential of rabbit antiserum to human C4 was detected after incubation with undiluted fetal calf serum. Preliminary studies indicate that C4 can also be detected on cultured human lymphoid cells by the binding of ^{125}I-labeled IgG of rabbit antiserum to human C4. The small number of C4 molecules on the cell membrane may explain why rabbit and goat antiserums to C4 did not kill lymphoid cells when reacted with cultured human lymphoid cells in conjunction with rabbit complement in the microlymphocytotoxic test (*12*). The lack of cytotoxicity of antiserums to C4 to cultured human lymphoid cells also indicates that the antiserums are not contaminated with antibodies to B-cell antigens (*13*).

Although C4 was detected on the surfaces of cells, we found no evidence for secretion of C4. Neither C4 hemolytic activity nor C4 protein was detected in exhausted culture media from RPMI 1788 or WI-L2 cells after 100-fold concentration.

Two approaches failed to reveal a relation between cell surface HLA antigens and C4. First, we mixed cells of the lymphoid cell lines RPMI 1788 (HLA-A2, -A10, -B7, and -B14) and WI-L2 (HLA-A1, -A2, -B5, and -BW17) with rabbit antiserum to C4 at the ratio of 2×10^6 cells per 25 μl of antiserum. After 2 hours at room temperature, the cells were washed twice with BSS and reacted in microcytotoxic tests (*12*) with HLA alloantiserums directed against HLA-A2, -B5, and -B7 and rabbit complement. No reduction in ability of the HLA alloantiserums to initiate complement-dependent lysis resulted. When RPMI 1778 and WI-L2 cells were similarly incubated with rabbit antibody to C4, their ability to specifically absorb HLA-A2, -B5, and -B7 alloantibodies was not impaired (*14*).

Second, 1 μl of alloantiserum directed to antigens of the locus A (HLA-A1, -A2, -A3, and -A9) and locus B (HLA-B5, -B7, and -B12) regions was incubated for 60 minutes at room temperature with 1 μl of phosphate-buffered saline (*p*H 7; ionic strength, 0.1) containing 1 to 2 μg of each of nine preparations of isolated C4. The alloantiserums were still able to lyse peripheral lymphocytes expressing the corresponding HLA specificities in the presence of rabbit complement (*14*). Parallel control studies showed that 0.001 μg of isolated HLA antigens (*15*) specifically reduced HLA antibody reactivity. No evidence of anti-C4 reactivity was shown by 10-μl portions of alloantiserums to HLA-A1, -A2, -B5, or -B7 since the sedimentation rate of 1 μg of ^{125}I-labeled C4 did not increase when centrifuged in sucrose density gradients. Finally, 2 μl of HLA alloantiserums did not reduce the hemolytic activity of 10 μg of C4.

In contrast, two of four goat antiserums and five rabbit antiserums to C4 inhibited the unidirectional MLR between allogeneic peripheral lymphocytes (as responder cells) and irradiated lymphocytes or cultured lymphoid cells (as stimulator cells). The results in Fig. 1A represent those obtained with peripheral lymphocytes from seven subjects responding to irradiated peripheral lymphocytes from five unrelated donors or to cultured human lymphoid cells WI-L2 or Raji. At final dilutions of 1 : 4 to 1 : 256 the various antiserums to C4 inhibited the MLR by 50 percent. However, antiserums to C4 did not change the kinetics of the response, which was totally blocked, as judged by [^{3}H]thymidine incorporation at 24-hour intervals during 6 days of observation (Fig. 1). Ten normal goat serums, 30 normal rabbit serums, and rabbit antiserums to human transferrin and albumin all failed to inhibit the MLR. Lysis of lymphoid cells was not responsible for the inhibition of the MLR since lymphocytes obtained from cultures after 6 days of incubation with antibody to C4 remained viable, as was judged by their ability to exclude trypan blue, and were not reduced in number as compared to control cultures.

Antiserums to C4 also inhibited the mitogenic response of peripheral lymphocytes to phytohemagglutinin (PHA) as shown in Fig. 1B. Antiserums to C4 were not directly mitogenic in the absence of PHA.

Our studies indicate that C4 is present on the surfaces of peripheral human lymphocytes and cultured human lymphoid cells. This is, to our knowledge, the first such report, although the mouse serum Ss protein [which acts as C4 in the murine complement system (*16*)] is expressed on murine cells (*17*), and receptors on the surfaces of human lymphoid cells absorb exogenously added human C4 (*18*). Although absorbed C4 could adhere to the surface of the peripheral lymphocytes used in our studies, this mechanism does not explain the presence of C4 on human lymphoid cells cultured for prolonged periods without human serum. Studies with antiserums to C4 provide no evidence of cross-reactivity between human C4 and components of fetal bovine serum. The synthesis of C4 and its preservation on the membranes of human lymphoid cells after several years in culture suggest that C4 subserves an essential cellular function. In view of the demonstrable link between genes controlling the expression of MHC antigens and genes regulating the serum levels of several complement components, inhibition of the MLR by antibody to C4 pointedly suggests that membrane-associated C4 functions in the afferent phase of immune recognition phenomena. This could occur by virtue

of a close spatial association of C4 with MLR determinants on the lymphoid cell surface. The existence of C4 in absence of MLR antigens on melanoma cells (unpublished results) indicates that C4 is not itself an MLR antigen. Alternatively, the MLR may require, in addition to differences in MLR antigens, other interactions or signals between the stimulating and responding cells. The C4, which in other systems functions as a ligand between complexes bearing C4 and lymphoid cells having C4 receptors, may operate equivalently in the MLR.

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Drinking Water and Cancer Mortality in Louisiana

Abstract. *Multivariant regression analysis indicates a statistically significant relation between cancer mortality rates in Louisiana and drinking water obtained from the Mississippi River. This is true for total cancer, cancer of the urinary organs, and cancer of the gastrointestinal tract.*

There is a growing consensus that the majority of human cancers are caused by chemical carcinogens in the environment and, hence, that they are ultimately preventable (*1*). Although there has been considerable interest in the role of air and food in environmental carcinogenesis, there has been relatively little attention directed to the possibility that carcinogens in drinking water may be causally related to human cancers (*2, 3*). Several studies have demonstrated the presence of chemical carcinogens in river water (*4*) and in treated municipal drinking water (*5, 6*), and others have shown that carcinogens are introduced during chlorine treatment (*7*); the question remains whether or not their concentrations, generally at or below the parts-per-billion level, are sufficiently high to have detectable effects on cancer rates. In 1974 we reported a preliminary regression analysis on the possible link between drinking water and cancer (*8*); we now present further results.

Louisiana was studied because of (i) suspicions of the possible etiologic role of drinking water in the high incidence of bladder cancer in New Orleans (*9*), (ii) the identification of carcinogens in Mississippi River drinking water (*10*), and (iii) the disparity between the quality of Mississippi River drinking water and other sources in Louisiana. Out of a total 1960 population of 3.3 million, living in 64 parishes, 32 percent (representing part or all of the population in 11 parishes) were supplied drinking water from the Mississippi River (Table 1), 56 percent were supplied groundwater, and 12 percent were supplied from other surface water supplies. On the assumption that concentrations of carcinogens in Mississippi River water have historically been higher than those in other water supplies in Louisiana, we defined the drinking water variable (*W*) as the percent of the parish population drinking water from the Mississippi River or its distributaries (*11*). Other independent variables are rurality (*R*), income (*I*), and occupation. Urbanization and income were defined for whites and nonwhites separately, but the other independent variables were not since the data were unavailable. The occupational variables are the proportion of the total number of employees of each parish in 1962 employed in the petroleum and coal products industry (*Pt*), in the chemicals industry (*C*), and in the mining industry (*M*) (*12*).

Table 1. Louisiana parishes that receive drinking water from the Mississippi River, given as percentage of the population of each parish.

Parish	Percent
Ascension	28
Assumption	83
Jefferson	100
Lafourche	96
Orleans	100
Plaquemines	100
St. Bernard	100
St. Charles	100
St. James	77
St. John the Baptist	81
St. Mary	61

Dependent variables were age-adjusted, 20-year mortality rates (*13*) associated with cancers of the gastrointestinal and urinary tracts (*14*) and with total cancer. Had the numbers of mortalities been sufficiently high, disaggregation into time periods shorter than 20 years and into age groups would have been desirable. However, for several cancer sites the number of mortalities over all age groups and over the entire 20-year period was small (sometimes zero) for some parishes.

To compensate for the small numbers, related cancer sites were grouped: for urinary cancers, the rates for kidney, bladder, and other urinary organs were combined; for gastrointestinal cancers, the rates for stomach, large intestine (except rectum), and rectum were combined. Although combining mortality rates tends, through averaging, to decrease random error, a regression with total cancer as the dependent variable may be hard to interpret because cancers of unrelated organs behave differently. Two sites were chosen that could be expected to be less linked to drinking water than urinary and gastrointestinal organs and in which cancers are moderately common, the breast for females and the prostate for males.

Little was known about the true interaction of the independent and dependent variables; therefore, because it is often robust against specification error, the simplest model, a linear one, was chosen and estimated by least squares (*15*). The coefficient for the source of drinking water in the regressions for gastrointestinal cancer is significant ($P < .01$) for all four