Brief Definitive Report

INDUCTION OF INTERLEUKIN 1 SECRETION AND ENHANCEMENT OF HUMORAL IMMUNITY BY BINDING OF HUMAN C5a TO MACROPHAGE SURFACE C5a RECEPTORS*

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Interaction of the human complement system with humoral and cellular immunity was first suggested by the observation that lymphocyte populations are separable on the basis of surface receptors for C3 (1). Subsequent reports indicated that isolated C3b either activates (2) or fails to activate (3) murine B lymphocytes. Additionally, C3c, C3d, and C3a have been implicated as regulators of the blastogenic (4) and antibody responses (5) of lymphocytes to antigen. In vivo complement activation in lymph nodes inhibits lymphocyte emigration for 24 h (6). Further studies indicate that depletion of circulating C3 with cobra venom factor, after priming, abrogates the development of B cell memory (7) as well as the cyclical antibody response (8). The use of antisera to purified complement components has supported a role for C4 in human (9) but not guinea pig models (10) of cellular immunity.

We have recently demonstrated the existence of specific receptors on murine macrophages for purified human C5a anaphylatoxin, a low molecular weight glycopeptide released from C5 during complement activation. These receptors have an apparent K_d of ~2 nM. In contrast, murine lymphoid cells are devoid of such receptors (11). Binding of C5a to macrophages results in augmentation of the primary humoral immune response (12). Immunopotentiation by C5a not only involves C5a receptor-bearing Ia^- accessory cells, but Ia^+ antigen-presenting cells as well. The present studies demonstrate that C5a induces macrophages and a macrophage cell line to secrete interleukin 1 (IL-1) but not IL-2 into the culture supernatant. Moreover, these supernatants augment the primary humoral response in a manner analogous to native C5a.

Materials and Methods

Mice. BALB/c and C3H/St male mice, 5–12 wk of age (from Scripps Clinic and Research Foundation) and CBA/CaJ and B6D2F$_1$ male mice, 8–12 wk of age (from The Jackson

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Laboratory, Bar Harbor, ME) were maintained on Wayne Lab-Blox F6 pellets and chlorinated water (pH 3.0).

Cell Lines. The cell lines P388 and P388D_1 were obtained through the Cell Distribution Center, Salk Institute for Biological Studies, La Jolla, CA. P388 and P388D_1 cells were maintained as described (11). CTLL cells were kindly provided by Dr. Amnon Altman, Medical Biology Institute, La Jolla, CA.

Preparation of Human C5a. Human C5a was isolated and quantitated as described previously (11). When assayed for endotoxin activity by the limulus lysate test, C5a was found to be at the lower limit of detection, containing less than or equal the amount of endotoxin in the culture medium itself.

Lymphocyte Cultures. The serum-containing medium used in these experiments has been described previously (12). Spleen cell suspensions were prepared in accordance with published procedures (13). For evaluation of the primary humoral immune response to sheep erythrocytes (SRBC), 10^7 murine spleen cells were cultured in 1.0 ml of 5% heat-inactivated (HI) fetal calf serum (FCS) containing medium for 4 d in the presence or absence of SRBC. Cells were incubated in culture trays (3008, Falcon Labware, Oxnard, CA) at 37°C in a humidified atmosphere of 10% CO_2 in air using tissue culture boxes rocked at 7 cpm. Cultures were fed daily with 50 μl of nutritional cocktail (13).

Pulse Treatment of Cells. P388 cells, P388D_1 cells, and splenic adherent cells were irradiated with 2,500 rad from a Gammacell 40 small animal irradiator (Atomic Energy of Canada, Ltd., Ottawa). Splenic adherent cells (SAC) were the cells remaining on plastic petri dishes after a 1 h incubation of spleen cells at 4×10^6/ml at 37°C and after three vigorous washes. These cells were exposed to 100 ng/ml C5a for 1 h, washed extensively, and cultured for 96 h in serum-free lymphocyte culture medium with 1 μM indomethacin. Cell lines were similarly pulsed with C5a and cultured at 2×10^6/ml for 96 h in lymphocyte culture medium containing 1% FCS and 1 μM indomethacin unless otherwise indicated.

Lymphokine Assays. IL-1 content was assayed by the thymocyte mitogenesis and costimulator assays (14, 15). Briefly, thymocytes from C3H/St mice, 5–7 wk of age, were cultured at 5×10^5/ml. Supernatants were used at a dilution of 1:4, and concanavalin (Con A) A at 3 μg/ml. After 66 h, cultures were pulsed with 1 μCi [^{3}H]thymidine ([^{3}H]TdR) for 6 h. IL-2 content was assessed by culturing the IL-2-dependent line CTLL, at 5×10^4/ml, with various dilutions of supernatant (16). Cultures were pulsed with 1 μCi [^{3}H]TdR for the final 24 h of the 2-d culture period.

Assayed of Plaque-forming Cells (PFC). PFC secreting antibodies against SRBC were evaluated after 4 d of culture using a modification (12) of the hemolytic plaque assay of Jerne and Nordin.

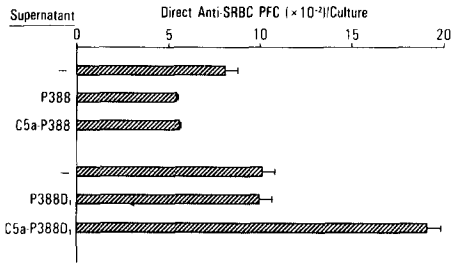
Results

Enhancement of the Primary Humoral Immune Response to SRBC by Supernatants from C5a-pulsed P388D_1 Cells or SAC. The ability of C5a and C5a-pulsed cells to potentiate the primary humoral immune response has been reported previously (12). The pertinent findings of those studies are summarized in Table I. Observation that pulse exposure of C5a receptor-bearing cells to C5a enhanced humoral immunity in vitro suggested that immunopotentiation might be mediated by secretion of a lymphokine.

TABLE I

Effect of Anaphylatoxins and C5a-pulsed Cells on the Primary Humoral Immune Response

Additive	Effect on primary SRBC response
C5a	Enhanced
$C5a_{des\ Arg}$	Enhanced
C3a	No effect
C5a-pulsed peritoneal cells	Enhanced
C5a-pulsed lymphocytes	No effect
C5a-pulsed P388D_1 cells	Enhanced



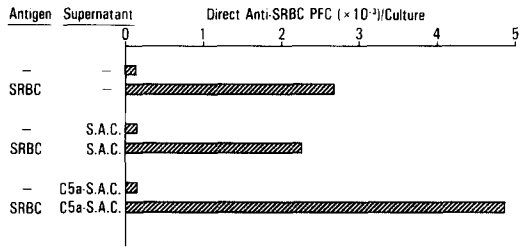


FIG. 1. (Left) Enhancement of the primary humoral immune reponse by supernatants from C5a-pulsed P388D1 and P388 cells. 10^7 viable spleen cells were cultured in 1.0 ml 5% FCS-containing medium for 4 d in the presence or absence of SRBC. Results are the mean of the difference of triplicate experimental and control (=152 + 22 PFC) cultures ± SE.

FIG. 2. (Right) Enhancement of the primary humoral immune response by supernatants from C5a-pulsed SAC. 10^7 viable spleen cells were cultured in 1.0 ml 5% FCS-containing medium for 4 d in the presence or absence of SRBC. Results are presented as the arithmetic mean of triplicate cultures.

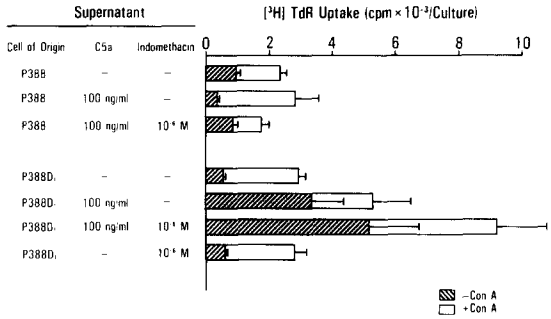


FIG. 3. IL-1 induction by C5a. Thymocytes from C3H/St mice, 5–7 wk of age, were cultured at 5×10^5/ml of medium containing 10% HI-FCS and 50 μM 2-mercaptoethanol. Supernatants were assayed at a 1:4 dilution, and Con A was added at 3 μg/ml. Cultures were pulsed with 1 μCi [^{3}H]TdR for the final 6 h of the 3-d incubation period. Results are presented as the arithmetic mean of five replicate cultures ± S.E.

To test this hypothesis, culture supernatants were generated from C5a-pulsed cells as detailed in Materials and Methods. Supernatants from the macrophage cell line P388, which does not bear C5a receptors, failed to enhance the response to antigen regardless of whether or not the cells had been exposed to C5a (Fig. 1). In contrast, supernatants from C5a receptor-bearing P388D1 cells pulsed with C5a significantly augmented the response to SRBC. Moreover, in parallel experiments with SAC, similar enhancement was observed (Fig. 2). The absence of increased PFC in control cultures (without antigen) clearly demonstrates that SAC supernatants, generated either with or without prior incubation with C5a, do not of themselves induce polyclonal immunoglobulin secretion.

Cellular Specificity of IL-1 Induction by C5a. Supernatants from C5a-pulsed cells were examined for content of the macrophage-derived lymphokine, IL-1. Two assays were used for this purpose: (*a*) enhancement of the Con A response of thymocytes, and (*b*) the mitogenic response of thymocytes to supernatant. Supernatants from P388 cells contained no IL-1 by either assay (Fig. 3). Culture supernatants from P388D1 cells, however, contained IL-1 as determined by both assays. This activity was even more pronounced when production of prostaglandins (which inhibit lymphocyte activation) was inhibited by 1 μM indomethacin.

TABLE II

Examination of Culture Supernatants for IL-2 Content

Supernatant		IL-2 content ([^{3}H]TdR uptake/10^3 CTLL)§
Cell of origin	Pulsed with:	
None	—	740 ± 65
P388D$_1$*	—	925 ± 20
P388D$_1$	C5a	910 ± 70
SAC‡	—	735 ± 35
SAC	C5a	775 ± 50
Rat spleen	Con A	5,500 ± 120

* 2×10^6 irradiated P388D$_1$ cells were pulsed with or without C5a for 1 h and cultured for 4 d.

‡ Residual adherent cells from 5×10^6 input CBA/CaJ spleen cells were irradiated and cultured for 4 d in serum-free medium with or without C5a.

§ 10^4 viable CTLL cells were cultured with a 1:8 dilution of the supernatant indicated. Cultures were pulsed with 1 μCi [^{3}H]TdR between 24 and 48 h. Results are expressed as the arithmatic mean of five replicate cultures ± SE, in cpm/10^3 CTLL to conform with the prevalent mode.

Examination of Culture Supernatants for IL-2 Content. Supernatants generated by either P388D$_1$ cells or splenic adherent cells in the presence or absence of C5a were tested for IL-2 content by their ability to promote proliferation of an IL-2-dependent cell line, CTLL (16). Neither P388D$_1$ cells nor SAC were induced to secrete IL-2 by C5a (Table II). Con A supernatants from rat spleen cells, used as a source of IL-2, served as a positive control.

Discussion

Human C5a anaphylatoxin binds to specific receptors found predominantly on Ia$^-$ murine macrophages (11). Its binding induces these cells to secrete an active principle that mediates enhancement of the primary humoral response to SRBC. These supernatants are unable to increase the number of spontaneous PFC to SRBC, indicating that immunoenhancement is antigen dependent and is not mediated by nonspecific polyclonal activation of B cells. Our current investigations clearly demonstrate that IL-1, but not IL-2, is selectively elaborated by these cells after a brief exposure to nanomolar concentrations of human C5a.

Identification of IL-1 in the supernatants of C5a-pulsed macrophages was accomplished by two different assays. The first, the thymocyte costimulator assay (15), detects both IL-1 and IL-2 activity. A second assay, involving induction of thymocyte mitogenesis (14), is selectively responsive to IL-1. The observation that supernatants from C5a-stimulated P388D$_1$ cells enhanced cellular responses in both assays implicates IL-1 as the putative mediator released from these cells. Moreover, when supernatants from C5a-pulsed P388D$_1$ and SAC were examined for IL-2 content by measuring proliferation of an IL-2-dependent cell line (CTLL), no IL-2 activity was demonstrable. Taken together, these results demonstrate that C5a promotes secretion of IL-1, but not IL-2, from responsive cells.

Several lines of evidence indicate that binding of C5a to specific macrophage receptors is the initiating event for elaboration of IL-1 by these cells. First, only cells that bear C5a receptors can secrete IL-1 in response to C5a stimulation. Cells that are devoid of C5a receptors consistently fail to produce this lymphokine after exposure to C5a. Furthermore, the absence of IL-1-like activity in the supernatants of C5a-pulsed

P388 cells militates against nonspecific carryover of cell-associated C5a as a trivial mechanism of augmentation. This interpretation is supported further by our observation that cell-bound C5a is rapidly degraded to biologically inactive amino acids and/or low molecular weight peptides (11). Finally, the inability of C5a to bind to murine lymphoid cells (11) makes it unlikely that any intact C5a that might dissociate from the macrophage receptor could directly account for the immunopotentiation observed.

Whereas the mechanism by which IL-1 enhances humoral immunity is controversial, the observation that humoral responses are augmented by IL-1 is well established (17). The two proposed target cells for IL-1 are the T cell, which may in turn produce IL-2 (18), and the B cell (19). Although investigation of the mode of action of IL-1 itself is beyond the scope of the current investigations, the data are compatible with either thesis.

Summary

The mechanism by which human C5a anaphylatoxin augments the primary humoral response of murine splenocytes to antigen has been investigated. Culture supernatants were generated from splenic adherent cells or macrophage cell lines after exposure to a brief pulse of human C5a. Supernatants from the macrophage-like cell line $P388D_1$, which bears surface receptors for C5a, enhance the PFC response to antigen, whereas those from the closely related cell line P388, which lacks surface receptors for C5a, fail to cause enhancement. Supernatants from splenic adherent cells, which also bear C5a receptors, similarly augment the SRBC response. Active supernatants, but not those devoid of activity, were shown to contain interleukin 1 (IL-1) activity by both the thymocyte mitogenesis and thymocyte costimulator assays. None of the supernatants contained IL-2 activity. These observations suggest that the recently described role of human C5a as an immunopotentiating modulator is mediated by its ability to induce production of IL-1 upon binding to specific receptors at the macrophage cell surface.

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