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

BY MICHAEL G. GOODMAN,‡ DENNIS E. CHENOWETH,§ AND WILLIAM O. WEIGLE

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037; and the Department of Pathology, M-012, University of California at San Diego, La Jolla, California 92093

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 Kd 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 B6D2F1 male mice, 8–12 wk of age (from The Jackson

* Supported in part by grants AI07007, AI18731, and AI15284 from the U. S. Public Health Service; Biomedical Research Support Grant RRO-5514 from the National Institutes of Health; and grant IM-42K from the American Cancer Society. Publication 2707 from the Department of Immunopathology, Scripps Clinic and Research Foundation.

‡ Recipient of Research Career Development Award AI00374 and grant AI 15284 from the U. S. Public Health Service.

§ Supported in part by Grant-in Aid 79-863 from the American Heart Association (San Diego Heart Association). Dr. Chenoweth completed this study as an Established Investigator of the American Heart Association.

912 J. Exp. Med. © The Rockefeller University Press • 0022-1007/82/09/0912/06 $1.00

Volume 1156 September 1982 912-917
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 P388D1 were obtained through the Cell Distribution Center, Salk Institute for Biological Studies, La Jolla, CA. P388 and P388D1 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 cpmin. Cultures were fed daily with 50 μl of nutritional cocktail (13).

Pulse Treatment of Cells. P388 cells, P388D1 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 × 10^6/ml. Supernatants were used at a dilution of 1:4, and concanavalin (Con A) at 3 μg/ml. After 66 h, cultures were pulsed with 1 μCi [3H]thymidine ([3H]Tdr) for 6 h. IL-2 content was assessed by culturing the IL-2-dependent line CTLL, at 5 × 10^5/ml, with various dilutions of supernatant (16). Cultures were pulsed with 1 μCi [3H]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 P388D1 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.

| Additive                              | Effect on primary SRBC response |
|---------------------------------------|---------------------------------|
| C5a                                   | Enhanced                        |
| C5a-pulsed SAC                        | Enhanced                        |
| C5a                                   | No effect                       |
| C5a-pulsed peritoneal cells           | Enhanced                        |
| C5a-pulsed lymphocytes                | No effect                       |
| C5a-pulsed P388D1 cells               | Enhanced                        |
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 | Cell of origin | Pulsed with | IL-2 content ($[^{3}H]$TdR uptake/10⁴ CTLL)§ |
|-------------|----------------|-------------|----------------------------------|
| None        | —              | —           | 740 ± 65                         |
| P388D₁*     | —              | C₅a         | 925 ± 20                         |
| P388D₁ C₅a | —              | —           | 910 ± 70                         |
| SAC         | C₅a            | —           | 735 ± 35                         |
| SAC         | —              | C₅a         | 775 ± 50                         |
| Rat spleen  | Con A          | —           | 5,500 ± 120                      |

* 2 × 10⁶ irradiated P388D₁ cells were pulsed with or without C₅a for 1 h and cultured for 4 d.
§ Residual adherent cells from 5 × 10⁶ input CBA/CaJ spleen cells were irradiated and cultured for 4 d in serum-free medium with or without C₅a.
§ 10⁴ 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 arithmetic mean of five replicate cultures ± SE, in cpm/10³ CTLL to conform with the prevalent mode.

**Examination of Culture Supernatants for IL-2 Content.** Supernatants generated by either P388D₁ 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 P388D₁ 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 P388D₁ cells enhanced cellular responses in both assays implicates IL-1 as the putative mediator released from these cells. Moreover, when supernatants from C₅a-pulsed P388D₁ 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 P388D1, 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.

The authors thank Mrs. Terry Else for superb technical assistance, Dr. Amnon Altman for providing us with the CTLL cell line, and Mrs. Alice Bruce Kay and Mrs. Barbara Marchand for excellent secretarial work.

References

1. Parish, C. R., and J. A. Hayward. 1974. The lymphocyte surface II. Separation of Fc receptor, C3 receptor and surface immunoglobulin-bearing lymphocytes. Proc. R. Soc. Lond. B Biol. Sci. 187:65.
2. Hartmann, K.-U., and V. A. Bokisch. 1975. Stimulation of murine B lymphocytes by isolated C3b. J. Exp. Med. 142:660.
3. Koopman, W. J., A. L. Sandberg, S. M. Wahl, and W. E. Mergenhagen. 1976. Interaction of soluble C3 fragments with guinea pig lymphocytes. Comparison of effects of C3a, C3b, C3c, and C3d on lymphokine production and lymphocyte proliferation. J. Immunol. 117:334.
4. Schenkein, H. A., and R. J. Genco. 1979. Inhibition of lymphocyte blastogenesis by C3c and C3d. J. Immunol. 122:1126.
5. Morgan, E. L., W. O. Weigle, and T. E. Hugli. Anaphylatoxin-mediated regulation of the
immune response. I. C3a-mediated suppression of human and murine humoral immune responses. *J. Exp. Med.* **155**:1412.

6. McConnell, I., and J. Hopkins. 1981. Lymphocyte traffic through antigen-stimulated lymph nodes. I. Complement activation within lymph nodes initiates cell shutdown. *Immunology.** **42**:217.

7. Klaus, G. G. B., and J. H. Humphrey. 1977. The generation of memory cells. I. The role of C3 in the generation of B memory cells. *Immunology.** **33**:31.

8. Romball, C. G., R. J. Ulevitch, and W. O. Weigle. 1980. Role of C3 in the regulation of a splenic PFC response in rabbits. *J. Immunol.** **124**:151.

9. Ferrone, S., M. A. Pellegrino, and N. R. Cooper. 1976. Expression of C4 on human lymphoid cells and possible involvement in immune recognition phenomena. *Science (Wash. D. C.).** **193**:53.

10. Burger, R., and E. M. Shevach. 1979. Evaluation of the role of C4 in the cellular immune response *in vitro*. *J. Immunol.** **122**:2388.

11. Chenoweth, D. E., M. G. Goodman, and W. O. Weigle. 1982. Demonstration of a specific receptor for human C5a anaphylatoxin on murine macrophages. *J. Exp. Med.** **156**:68.

12. Goodman, M. G., D. E. Chenoweth, and W. O. Weigle. 1982. Potentiation of the primary humoral immune response *in vitro* by C5a anaphylatoxin. *J. Immunol.** **129**:70.

13. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures in normal mice. *J. Exp. Med.** **126**:423.

14. Mizel, S. B., J. J. Oppenheim, and D. L. Rosenstreich. 1978. Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D1. *J. Immunol.** **120**:1497.

15. Melzer, M. S., and J. J. Oppenheim. 1977. Bidirectional amplification of macrophage-lymphocyte interactions: enhanced lymphocyte activation factor production by activated adherent mouse peritoneal cells. *J. Immunol.** **118**:77.

16. Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.** **120**:2027.

17. Farrar, J. J., and M. L. Hilfiker. 1982. Antigen-nonspecific helper factors in the antibody response. *Fed. Proc.** **41**:263.

18. Farrar, J. J., and W. J. Koopman. 1979. Characterization of mitogenic factors and their effect on the antibody response *in vitro*. In *Biology of the Lymphokines*. S. Cohen, E. Pick, and J. J. Oppenheim, editors. Academic Press, Inc., New York. 325–346.

19. Wood, D. D., and P. M. Cameron. 1976. Stimulation of the release of a B cell-activating factor from human monocytes. *Cell. Immunol.** **21**:133.