The Journal of Immunology

RESEARCH ARTICLE | NOVEMBER 01 1978

Studies on Immunosuppression by Cobra Venom Factor: III. On Early Responses to Sheep Erythrocytes in C5-Deficient Mice[1] FREE

Giorgio P. Martinelli; ... et. al

J Immunol (1978) 121 (5): 2052–2055.
https://doi.org/10.4049/jimmunol.121.5.2052

0022-1767/78/1215-2052$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 121, No. 5, November 1978
Printed in U.S.A.

STUDIES ON IMMUNOSUPPRESSION BY COBRA VENOM FACTOR

III. On Early Responses to Sheep Erythrocytes in C5-Deficient Mice[1]

GIORGIO P. MARTINELLI,[2] TAKEMASA MATSUDA, HARRIET S. WAKS, AND ABRAHAM G. OSLER

From the Department of Medical Immunology, The Public Health Research Institute of The City of New York, Inc., New York 10016

CVF administered before immunization can profoundly depress humoral responses in C-sufficient mice. In AKR/J C5$^-$ mice given CVF before i.v. immunization with SRBC, only IgG levels were depressed, IgM titers being equivalent to those of untreated controls. The immunosuppressive effect became inapparent when the i.p. route of immunization was adopted. In DBA/2J C5$^-$ mice reduction of both IgG and IgM titers was observed irrespective of the route of immunization. The degree of suppression was, however, much more marked when mice were challenged intravenously. Essentially identical results were obtained with C5$^+$ DBA/1J mice. These studies indicate that immunosuppression by CVF is unrelated to activation of the late C components. The significance of these findings is discussed with reference to the possibility that the generation of biologically active C fragments in conjunction with a C3 deficiency may account for immunosuppression by CVF.

Recent reports from several laboratories indicate that early antibody formation in mice is substantially reduced after the injection of the anticomplementary glycoprotein, cobra venom factor (CVF)[3] (1–3). Studies in this laboratory, based on IgG antibody estimates in weight units, have confirmed these findings for a number of T-dependent antigens (3a). We have also demonstrated that the immunosuppressive activity of CVF is equally manifest for the response of T-independent antigens (3b). The latter observation is particularly relevant to an understanding of the mechanism of CVF action in immunosuppression. It cannot be reconciled with the views that the diminished antibody production results solely from an impaired contribution of the complement (C) system to cell cooperation, a defect attributed to the C3 deficiency after CVF administration (1–3). Since C3 depression is brought about by systemic C activation, we have previously suggested that the generation of biologically active C fragments may also be part of the immunosuppressive mechanism by virtue of their action on macrophages (3a, 3b). Exposure of these cells to C-cleavage products might lead to changes in their state of activation and secretory functions (4–7). The experiments detailed below were designed to ascertain which step in the C-activation sequence might be meaningfully associated with the limitation in antibody formation.

Received for publication June 2, 1978.
Accepted for publication August 18, 1978.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Support was received from American Cancer Society Grant IM-23N and United States Public Health Service Grant AI08710.
[2] Address all correspondence to G. P. M. The New York Blood Center, Dept. of Immunogenetics, 310 East 67th Street, New York 10021.
[3] Abbreviations used in this paper: AH50, hemolytic titers expressed as the reciprocal of the serum dilution yielding 50% lysis; CFAA, complement fixation antibody assay; C5$^-$, C5 deficient; C5$^+$, C5 sufficient; CR, complement receptor; CVF, cobra venom factor; dPFC, direct plaque forming cells; n.s., not significant, $p > 0.05$.

MATERIALS AND METHODS

Animals. Male AKR/J, DBA/2J, and DBA/1J mice 8 to 10 weeks of age were purchased from the Jackson Laboratories, Bar Harbor, Maine. AKR/J and DBA/2J are C-deficient strains lacking C5 (8, 9).

Immunogen. SRBC were obtained from extensively washed sheep blood preserved in Alsever's solution (Colorado Serum Co., Denver, Colo.). Suspensions were standardized to contain 1×10^9 cells per milliliter and diluted in PBS as indicated for immunization.

Decomplementation and immunization. C depletion was achieved by i.p. injection of *Naja naja* CVF (Cordis Labs., Miami, Fla.) as described in (10). Twenty-five units were administered divided in three or four equal doses over a period of 24 hr. Sheep erythrocytes were administered 1 hr after the last injection of CVF at which time C3 levels were less than 5% of the normal values as judged by radial immunodiffusion assays with sera of all three strains of mice.

Assays for hemolytic antibodies. These titrations were carried out as described in Reference 3a.

Weight estimates of IgG antibodies by the complement assay (CFAA). This assay yields quantitative weight estimates of IgG antibodies to sheep erythrocytes and is fully described in (11).

Plaque forming cell (PFC) assays. Assays for direct plaque forming cells (dPFC) were carried out by the method of Cunningham and Szenberg (12).

RESULTS

The effect of CVF on the anti-SRBC response of AKR/J, C5$^-$ mice. These experiments were undertaken to ascertain whether activation of C5 and of the late components constitute part of the mechanism of immunosuppression. The C5$^-$ mice seemed particularly well suited for this purpose because if antibody formation were indeed reduced in these mice, an additional insight into the mechanism would be achieved. Preliminary experiments indicated that, as in C5$^+$ mice (3a, 3b), the injection of 25 units of CVF depleted C3 levels by 95% or more for at least 72 hr. The data in Table I summarize the results of two experiments in which the early response of C5$^-$ mice to SRBC was evaluated after administration of CVF. The results show that whereas the IgG response was suppressed, the

IgM response assayed in terms of dPFC was unaffected. The latter conclusion stems also from the results of the hemolytic antibody titrations that detect IgM antibodies preferentially.[4] These findings differ from those obtained previously with BALB/c mice since in the latter case both Ig classes were diminished by CVF treatment (3a). The experiments in Table I were repeated with one change: the AKR/J mice received an i.p. rather than an i.v. injection of 10^8 SRBC. As is apparent from the data in Table II, the i.p. route of immunization nullified the immunosuppression by CVF. A second variable was therefore explored; namely, the number of SRBC used to immunize the mice was reduced. As may be noted in Table III, the response to 3×10^7 was unaffected by CVF, whereas the IgG response to 1×10^7 was substantially suppressed. The AH50 titers were equivalent in the CVF-treated and control mice for all three SRBC doses.

Effect of CVF on the anti-SRBC response of C5⁻ DBA/2J mice and of C5⁺ DBA/1J mice. In order to establish whether these observations were unique for the AKR/J strain, we repeated these experiments in DBA/2J mice, which are also C5 deficient (8, 9). The controls used in these experiments were mice of the DBA/1J ($C5^+$) strain, which is related to the DBA/2J. The results of these experiments are shown in Table IV, which indicate that essentially similar degrees of suppression of both the IgG and IgM responses occurred in the $C5^+$ and $C5^-$ mice. It is also apparent that the depressive effect of CVF was more pronounced after immunization by the i.v. route with virtually complete abrogation of the IgG antibody response. These results show a trend similar to that obtained with the AKR/J mice in demonstrating that the degree of antibody suppression associated with CVF pretreatment varies with the route of antigen injection. Of further interest is the finding that similar antibody levels were obtained in both DBA strains indicating that the C5 deficiency in the DBA/2J mice does not impair their ability to mount a humoral response to SRBC.

TABLE I

The effect of CVF on the response of AKR/J mice to an i.v. injection of 10^8 SRBC[a]

Expt.	CVF Units	Day	m̄ dPFC/ Spleen ± S.D. (10^{-3})	m̄ AH50 Titers ± S.D.	m̄ IgG Antibody Levels[b]	
					μg/ml ± S.D.	
A	25	5	29.6 ± 6.3	3637 ± 860	7.5 ± 1.4	$p < 0.001$
	PBS	5	35.9 ± 9.9	4317 ± 528	17.6 ± 3.1	
B	25	5		1872 ± 621	5.5 ± 2.9	$p < 0.001$
	PBS	5		2554 ± 500	20.3 ± 4.0	
	25	8		1954 ± 261	15.1 ± 3.5	$p < 0.01$
	PBS	8		2028 ± 236	31.3 ± 10.7	

[a] There were six to seven mice in each group.
[b] Significant suppression is indicated by a p value.

TABLE II

The effect of CVF on the response of AKR/J mice to an i.p. injection of 10^8 SRBC[a]

Expt.	CVF Units	Day	m̄ dPFC/ Spleen ± S.D. (10^{-3})	m̄ AH50 Titers ± S.D.	m̄ IgG Antibody Levels
					μg/ml ± S.D.
A	25	5	80.0 ± 8.3	3912 ± 407	7.6 ± 1.6
	PBS	5	55.4 ± 16.3	3864 ± 594	10.1 ± 2.3
	25	8	4.6 ± 2.0	2225 ± 420	51.5 ± 13.7
	PBS	8	6.1 ± 1.1	1668 ± 225	58.6 ± 7.4
B	25	8		1655 ± 329	45.4 ± 15.0
	PBS	8		1369 ± 224	47.1 ± 29.0

[a] There were six to seven mice per group.

TABLE III

Effect on the day 8 response of AKR/J mice to an i.p. injection of 3×10^7 or 1×10^7 SRBC[a]

CVF Units	SRBC Dose	m̄ AH50 Titers ± S.D.	m̄ IgG Antibody Levels[b]	
			μg/ml ± S.D.	
25	3×10^7	1195 ± 655	34.6 ± 16.7	
PBS	3×10^7	1759 ± 486	54.0 ± 18.0	
25	1×10^7	1560 ± 615	18.5 ± 12.0	$p < 0.025$
PBS	1×10^7	1694 ± 150	56.6 ± 25.6	

[a] There were six mice per group.
[b] Significant differences are indicated by a p value.

DISCUSSION

The experiments described above extend present knowledge regarding the immunosuppressive effect of CVF in the demonstration that the late C components do not materially affect this phenomenon. The validity of this conclusion emerges from the data in Table IV, which are noteworthy in two respects. The ability of the $C5^-$ DBA/2J mice to mount an immune response is equivalent to that of their $C5^+$ counterparts. Second, the extent of immunosuppression associated with CVF is of the same magnitude in both the $C5^+$ and $C5^-$ mice and involves both the IgG and IgM antibody classes. Although the latter is inferred from hemolytic antibody titers, there is little doubt that this activity is largely, if not entirely, due to IgM immunoglobulins.[3] The antibody weight estimates are unequivocal in this regard.

It therefore appears that immunosuppression by CVF does not involve utilization of C5 and the assembly of the late components. Since CVF *per se* does not seem to affect lymphoid cell function directly (2, 3a, 14), its activity can be attributed to activation and/or depletion of C components. It has been proposed that depletion of C3 impairs cell cooperation and this leads to immunosuppression (1–3), a view prompted by the finding that C activation does not appear to affect the function of T cells, the number of CR^+ lymphocytes, or the immune response to certain T-independent antigens (1–3, 13). As noted previously (3a), acceptance of this model is subject to several reservations since it is based on the inference that C3 receptors are instrumental in cell cooperation and that a C3 deficiency is critically involved in immunosuppression. We have shown that the latter two events can be dissociated by such means as the use of CFA (3a), the route of immunization, and the dose of the immunogen (Tables II and III). Further, the immune response to T-independent antigens, which presumably occurs without cell cooperation (*cf,* however, 14, 15), can be readily depressed by CVF administration (3b). Moreover, direct evidence for a role of C3 receptors in cell cooperation is as yet unavailable. Nor have the functional attributes of CR^+ or CR^- lymphocytes been fully characterized (16). The present results are also not explicable simply in terms of a C deficiency. Thus, the data in Tables II and III indicate that antibody levels in AKR/J mice immunized i.p. may not be affected after profound C3 depletion. Only when the lowest number of SRBC was injected did immunosuppression follow, and this was limited to the IgG com-

[4] Osler, A. G. On the precedence of 19S antibodies in the early immune response. Immunochemistry. Accepted for publication.

TABLE IV

Effect of CVF on the day 8 response of DBA/1J and DBA/2J[a] mice to i.v. or i.p. challenge with 10^8 SRBC[b,c]

CVF Units	Route of Immunization	m̄ AH50 Titers ± S.D.		Suppression (%)	m̄ IgG Antibody Levels		Suppression (%)
					μg/ml ± S.D.		
				DBA/1J			
25	i.v.	257 ± 121	$p < 0.001$	87.3	1.2 ± 0.2	$p < 0.001$	96.5
PBS	i.v.	3611 ± 366			34.1 ± 14.3		
25	i.p.	1274 ± 439	$p < 0.001$	52.0	16.8 ± 6.1	$p < 0.001$	65.3
PBS	i.p.	2652 ± 422			48.4 ± 8.8		
				DBA/2J[a]			
25	i.v.	691 ± 128	$p < 0.001$	83.7	1.7 ± 0.6	$p < 0.001$	92.8
PBS	i.v.	5424 ± 407			23.8 ± 4.6		
25	i.p.	2298 ± 626	$p < 0.001$	44.8	16.6 ± 5.6	$p < 0.001$	66.9
PBS	i.p.	4166 ± 604			50.2 ± 5.1		

[a] C5 deficient.
[b] There were six mice per group.
[c] This table comprises a single experiment.

ponent. In contrast, IgG antibody formation was depressed after an i.v. challenge with as many as 10^8 SRBC. Greater immunosuppression after i.v. as compared to i.p. injection of SRBC is also shown in Table IV for the two strains of DBA mice. Both the IgG and IgM responses to i.v. immunization are almost completely abrogated by CVF treatment. The data presented previously (3a, 3b) together with the present findings suggest to us that the mechanism of action of CVF is of a multifactorial nature. The reduction of C3 levels may influence the immune response by altering the C-dependent binding of immune complexes to immunologically relevant sites (17). That an intact C system may be an important factor in antigen trapping and/or processing is also supported by recent findings of depressed antibody responses in C4-deficient guinea pigs (18). On the basis of the present knowledge it cannot, however, be excluded that CVF-mediated immunosuppression may also result from the biologic activities of systemically generated C fragments. In view of the mounting evidence for an essential role of macrophages in mediating immune responses *in vitro* and for the regulation of their state of activation by C fragments (4–7), their auxiliary function in the induction of antibody synthesis could be hampered by systemic C activation as discussed in (3a, 3b). The validity of these hypotheses requires the demonstration that C-cleavage products such as Bb can indeed modulate cellular activities and humoral responses, an inference amenable to further exploration.

REFERENCES

1. Pepys, M. B. 1974. Role of complement in induction of antibody production *in vivo*. J. Exp. Med. 140:126.
2. Pepys, M. B. 1976. Role of complement in the induction of immunological responses. Transplant. Rev. 32:93.
3. Lewis, G. K., R. Ranken, and J. W. Goodman. 1977. Complement dependent and independent pathways of T-B cell cooperation. J. Immunol. 118:1744.

3a. Martinelli, G. P., T. Masuda, and A. G. Osler. 1978. Studies of immunosuppression by cobra venom factor. I. On early IgG and IgM responses to sheep erythrocytes and DNP-protein conjugates. J. Immunol. 121:2043.

3b. Matsuda, T., G. P. Martinelli, and A. G. Osler. 1978. Studies of immunosuppression by cobra venom factor. II. On responses to DNP·Ficoll and to DNP·polyacrylamide. J. Immunol. 121:2048.

4. Bianco, C., A. Eden, and Z. A. Cohn. 1976. The induction of macrophage spreading: role of coagulation factors and the complement system. J. Exp. Med. 114:1531.
5. Bianco, C., O. Götze, and Z. A. Cohn. 1977. Regulation of macrophage mobility by components of the complement system. J. Immunol. 120:1765.
6. Götze, O., C. Bianco, and Z. A. Cohn. 1977. The induction of macrophage spreading by factor B of the properdin system. J. Immunol. 120:1776.
7. Schorlemmer, H. U., and A. C. Allison. 1976. Effects of activated complement components on enzyme secretion by macrophages. Immunology 31:781.
8. Cinader, B. S., Dubiski, and C. Wardlaw. 1964. Distribution, inheritance and properties of an antigen, MuBl and its relation to hemolytic complement. J. Exp. Med. 120:897.
9. Nilsson, M. R., and H. J. Müller-Eberhard. 1967. Deficiency in the fifth component of complement in mice with an inherited complement defect. J. Exp. Med. 125:1.
10. Cochrane, C. G., H. J. Müller-Eberhard, and B. S. Aikin. 1970. Depletion of plasma complement *in vivo* by a protein of cobra venom: its effects on various immunologic reactions. J. Immunol. 105:55.
11. Martinelli, G. P., and A. G. Osler. 1978. The application of complement fixation to antibody weight estimates. II. To sheep erythrocytes. Ann. Immunol. Inst. Pasteur 129C:323.
12. Cunningham, A. J., and A. Szenberg. 1968. Further improvement in the plaque technique for detecting single antibody forming cells. Immunology 14:599.
13. Pepys, M. B., D. D. Mirjah, A. C. Dash, and M. H. Wansborough-Jones. 1976. Immunosuppression by cobra factor: distribution, antigen induced blast transformation and trapping of lymphocytes during *in vivo* complement depletion. Cell. Immunol. 21:327.
14. Klaus, G. G. B., J. M. Phillips, J. H. Humphrey, D. W. Dresser, and A. Cross. 1976. The immunological properties of haptens coupled to thymus independent carrier molecules. IV. The IgG response to dinitrophenylated Ficoll. Eur. J. Immunol. 6:429.
15. Lee, K. C., Shiozawa, A., Shaw, and E. Diener. 1976. Requirements for accessory cells in the antibody response to T cell-independent antigens *in vitro*. Eur. J. Immunol. 6:63.
16. Simon, M., and U. Hämmerling. 1977. Functional analysis of B cells subpopulations: responsiveness of phenotypically characterized

CR^+ and CR^- B cells subsets to a T dependent antigen *in vitro*. J. Immunol. 119:1700.

17. Miller, G. W., and V. Nussenzweig. 1974. Complement as regulator of interactions between immune complexes and cell membranes. J. Immunol. 113:464.
18. Ochs, H. D., C. G. Jackson, M. M. Frank, S. W. Hosea, and R. J. Wedgwood. 1978. Antibody response to a T cell dependent antigen in C4 deficiency. J. Immunol. 120:1789.