Induction of Immune Response to Plasma Lipoproteins with C-Reactive Protein

P. G. Nazarov, I. V. Petrov, L. S. Kositskaya, L. K. Berestovaya, and A. D. Denisenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 7, pp. 76-79, July, 1998
Original article submitted May 28, 1997

Purified C-reactive protein induces the production of autoantibodies to lipoprotein B-containing lipoproteins in animals. This is due to a C-reactive protein idiotype that permits the interference of C-reactive protein in the idiotype-anti-idiotypical immunological reactions and stimulation of autoimmune response to lipoproteins.

Key Words: *C-reactive protein; low- and very low-density lipoproteins; autoantibodies; idiotype-anti-idiotypic regulation*

Inflammatory processes often involve shifts in the plasma lipoprotein (LP) spectrum [5,11]. C-reactive protein (CRP), a factor of acute phase of inflammation, is involved in LP metabolism. Binding to low- (LDLP) and very low-density LP (VLDLP) [10], CRP activates their take-up by macrophages [6] and promotes their accumulation in arterial walls [8,9]. Due to affinity for phosphorylcholine, CRP idiotype is similar to antibodies to it [12] and simulates their capacity of selective regulation of immune response to phosphorylcholine-containing antigens [7]. CRP may be involved in autoimmune reaction to LP. The role of CRP in specific regulation of immune response to LP is not clear.

We investigated the effect of CRP on humoral immune response to LP.

MATERIALS AND METHODS

Native human pentamer CRP (pCRP) or its monomers (mCRP), CRP immune complex with asinine IgG antibodies, human LP, or control preparations (phosphate-buffered saline, pH 7.2, IgG and human serum albumin, or human IgG) with complete Freund's adjuvant during the first 2-3 weeks and then without it were twice injected to two-month-old male CBA/CaJ mice and Chinchilla rabbits from Rappolovo Breeding Center. To rabbits the first dose (300 μg protein) was injected in the hind paw pads and the second (100 μg) in the popliteal lymph nodes; to mouse both doses (100 μg protein) were injected intraperitoneally. We used electrophoretically homogeneous pCRP [2] free from apolipoprotein B-containing LP (according to agarose and cellulose acetate electrophoresis, gradient electrophoresis with SDS, rocket immunoelectrophoresis, immunoblotting, and passive hemagglutination inhibition test with rabbit antibodies to human apolipoprotein B). Lipoproteins were isolated from human and animal plasma by sequential ultracentrifugation [4] and analyzed for the presence of CRP in passive hemagglutination test with commercial asinine antiserum to human pCRP and rabbit antisera to human pCRP and mCRP [2] exhausted with human VLDLP. Guinea pig antibodies to rabbit apolipoprotein B and rabbit antibodies to human LDLP and VLDLP and the corresponding anti-idiotypic sera were used [1]. LDLP-specific antibody-producing cells were detected in mouse spleens by Jerne's method [3]. Circulating antibodies to LP, CRP, and control proteins were detected by passive hemagglutination test and anti-idiotypic antibodies by passive hemagglutination inhibition test.

Departments of Immunology and Biochemistry, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg

0007-4888/98/0007-0708$20.00

RESULTS

Injection of pCRP and mCRP induced the production of antibodies to CRP and to heterologous LDLP and VLDLP (Table 1). Injection of buffered saline and IgG with and without complete Freund's adjuvant produced no effect of this kind. Moreover, pCRP and mCRP induced the production of autoantibodies to LP. Antibodies to homologous LDLP (Table 1) were detected in rabbit sera (Table 1). Cells producing IgM and IgG antibodies to syngeneic LP were found in mouse spleen (Table 2). Thus, the immune response to LP induced by CRP can be regarded as autoimmune.

Bispecific antibodies to CRP and LP appeared after injection of LP. On day 10 after the second injection of 10 μg human LDLP with complete Freund's adjuvant to mice, antibody titers were (ln): 12.4±0.5 to human LDLP (n=9) and 4.7±1.8 to CRP (n=9).

Probable causes of production of anti-LP antibodies in response to CRP are CRP contamination by LP, polyclonal activation of immune system with CRP, immunoregulatory effect of CRP idiotype, or modification of animal LP as a result of their interaction with CRP.

Testing of CRP for LP traces gave negative results, the sensitivity of methods permitting the detection of 20±10 ng apolipoprotein B per mg CRP. Lipoprotein admixture was below this threshold, while with injection of 100-300 μg CRP the animals could get no more than 2-6 ng apolipoprotein B. Such LP doses did not induce immune response in mice. Anti-LP antibodies were detected after a single injection of at least 10 μg LDLP or two injections of at least 1 μg, which is 200-500 times more than could be in CRP preparations. Therefore, the capacity of CRP to induce immune response to LP was not due to an admixture of LP.

Unlike mCRP, pCRP is mitogenic [2]. On the other hand, both CRP forms effectively induced immune response to LP but not to other antigens (sheep erythrocytes, human, rabbit, mouse, and asinine IgG, Table 1). Moreover, combined injection of pCRP and pure LDLP did not enhance the immune response to LP (Table 3). Therefore, the induction of antibodies to LP in response to CRP cannot be explained by CRP mitogenicity and polyclonal activation of immune system.

The following data indicate that immune response to LP after injection of CRP is caused by CRP intervention in the idiotypical regulation of immunogenesis.

First, CRP possesses an idiotype related to the idiotype of anti-LP antibodies: rabbit anti-idiotypic serum specific to homologous antibodies to human LDLP, not reacting with LP and rabbit antibodies to VLDLP and LDLP, reacted with human mCRP in the passive hemagglutination inhibition test (1:64).

TABLE 1. Spectrum of Antibodies Induced by CRP ($M\pm m$)

Immunization	n	Level of antibodies to antigens, $\log_2$ of titers									
		pCRP	mCRP	human VLDLP	human LDLP	rabbit LDLP	mouse LDLP	sheep erythrocyte	human IgG	rabbit IgG	asinine IgG
Mice											
pCRP	9	0		7.8±0.4	12.0±0.6			0	0	0	0
pCRP+CFA	18	3.5±0.3		10.8±0.5	13.3±0.4	3.3±0.5	0	0	0	0	0
mCRP+CFA	9	2.2±1.4			12.2±0.8	3.3±±0.5	0				
IC	9	0		0	0			0	0	0	0
IC+CFA	9	10.3±0.2		0	0			0	0	0	0
Human IgG	9	0		0	0			0	0	0	0
Human IgG+CFA	9	0		0	0			0	9.3±0.1	0	0
BNS	9	0		0	0			0	0	0	0
BNS+CFA	9	0		0	0	0	0	0	0	0	0
Rabbits											
pCRP+CFA	2	12.9±0.5	3.5±0.4		11.9±0.4	12.1±2.2	8.1±1.8				
mCRP+CFA	2	6.9±1.9	10.0±1.0		10.1±1.2	10.1±1.2	8.4±3.5				
Human IgG+CFA	2	0	0		0		0				

Note. CFA: complete Freund's adjuvant; IC: immune complex; BNS: buffered normal saline.

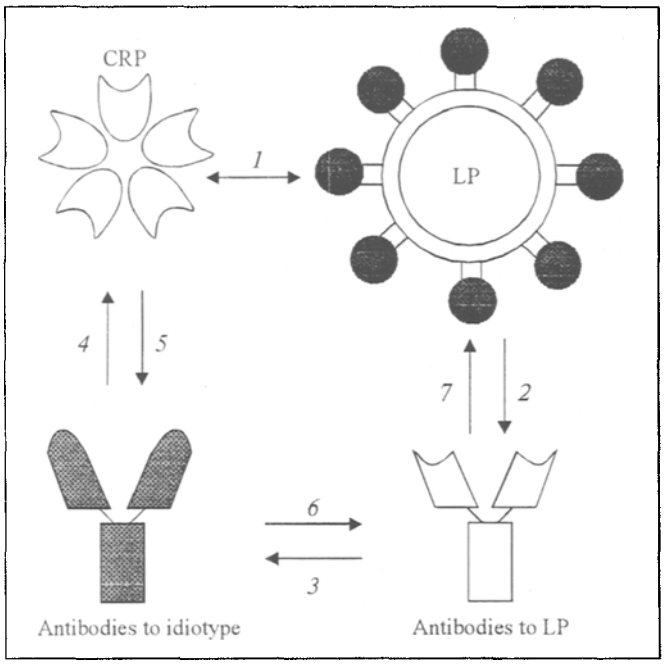

Fig. 1. A scheme of autoimmune reactions against plasma lipoprotein (LP) induced by C-reactive protein (CRP). First pathway: *1*) CRP binding to LP leads to antigenic modification of LP and accelerates their clearance; 2) CRP-modifical LP become autoantigen, which leads to induction of production of antibodies to LP; *3*) antibodies to LP induce the production of anti-idiotypic antibodies; *4*) anti-idiotypic antibodies react with CRP (and induce its production ?). Second pathway: *5*) CRP induces the production of antibodies to an idiotype common for CRP and anti-LP antibodies; *6*) anti-idiotypic antibodies induce the production of anti-anti-idiotypic antibodies (i. e. antibodies to LP); *7,1*) CRP and antibodies to LP bind to LP, which leads to repetition of stages 2, 3, and 4. Dark circles on LP corpuscle are the determinants cross-reacting with CRP and antibodies to LP.

Second, anti-idiotypic antibodies reacting with syngeneic antibodies to human LDLP were detected in mice injected with CRP. Anti-idiotypic antibodies were searched for in mice without direct antibodies to LP which were injected with pCRP as a component of immune complex, human IgG, or buffered normal saline. Four pools of sera from mice immunized with pCRP once and twice with and without complete Freund's adjuvant were used as agglutinating reagents in the passive hemagglutination inhibition test. Positive results indicating the presence of anti-idiotypic antibodies were obtained in mice injected with pCRP in a single dose with complete Freund's adjuvant. Immune complexes induced a stronger anti-idiotypic response than free antigen, which may account for the narrow range of anti-idiotypic antibodies.

Thus, CRP simulates the immunogenic properties of LP, because it is an LP-binding protein carrying an idiotype which makes it similar to anti-LP antibodies. This idiotype is recognized by the clone of immunocompetent cells possessing relevant antigen-recognizing receptors; the recognition leads to receptor activation and development of anti-idiotypic immune response. The clone may be cross-activated by both CRP and antibodies to LP. Antibodies to a common idiotype are the product of this clone. They activate another cell clone and induce the production of second-order antibodies that react with the determinants of apolipoprotein B-containing LP and are detected as anti-LP antibodies. Therefore, the production of anti-LP antibodies under the effect of CRP is a result of two-stage immune response regulated through an idiotype-anti-idiotypic relationship (Fig. 1).

Modifications of animal plasma LP resulting from their reactions with injected CRP might contribute to induction of immune response to LP. However, the causes of an immune response higher to heterologous LP than to autologous one, particularly in mice, are not clear.

Infective agents (bacteria, protozoa, etc.) containing phosphorylcholine can form an immune background represented by at least two cell populations: specific (idiotype-positive) and the corresponding anti-idiotypic. The latter is activated by any molecule carrying a relevant idiotype, including CRP, which leads to production of antibodies reacting with phosphorylcholine and LP. The role of

TABLE 2. Production of Antbodies to Human LDLP and Autoantibodies to Syngeneic LDLP by Splenocytes of Mice Immunized with pCRP with Complete Freund's Adjuvant (Number of Antibody-Producing Cells/Spleen, *M±m*)

Specificity	Isotype	Mouse immunization		
		buffered normal saline (*n*=8)	pCRP (*n*=8)	human LDLP (*n*=8)
Human LDLP	IgM	147±30	529±82*	4612±1037*+
	IgG	262±21	4320±666*	8078±1260*+
Mouse LDLP	IgM	18±8	76±18*	7±5*
	IgG	220±42	738±26*	202±128*

Note. *$p<0.05$ *vs.* animals injected with normal saline, *+$p<0.01$ *vs.* animals injected with pCRP.

TABLE 3. Immune Response of Mice to LDLP Injected in Complex with Human LDLP (*M*±*m*)

Preparation injected with LDLP and complete Freund's adjuvant	Number of injections	Level of antibodies to human LDLP, In	
		day 7	day 10
Buffered normal saline	1	5.6±1.0	11.5±1.1
Human serum albumin	1	5.2±1.9	7.0±2.0
pCRP	1	5.7±1.3	10.9±0.5
Buffered normal saline	2	13.9±0.1	13.5±0.8
Human serum albumin	2	13.8±0.5	14.5±0.6
pCRP	2	14.2±0.2	14.5±0.6

CRP-dependent immunological reactions in the pathogenesis of atherosclerosis remains to be studied.

REFERENCES

1. L. S. Kositskaya, B. N. Sofronov, and T. G. Shemerovskaya, *Immunologiya,* No. 3, 16-17 (1990).
2. P. G. Nazarov, A. V. Polevshchikov, L. K. Berestovaya, *et al., Byull. Eksp. Biol. Med.,* **116,** No. 12, 609-611 (1993).
3. N. K. Jerne, C. Henry, and A. A. Nordin, in: *Methods in Immunology and Immunochemistry,* eds. C. A. Williams *et al.,* New York (1976), Vol. 5, pp. 335-370.
4. F. T. Lindgren, in: *Analysis of Lipids and Lipoproteins,* New York (1975), pp. 204-224.
5. A. D. Mbewu, P. N. Durington, S. Bulleid, and M. I. Mackness, *Atherosclerosis,* **103,** 65-71 (1993).
6. S. Mookerjea, J. Francis, D. Hunt, *et al., Arterioscler. Thromb.,* **14,** 282-287 (1994).
7. S. Nakayama, T. W. duClos, H. Gewurz, and C. Mold, *J. Immunol.,* **132,** 1336-1340 (1984).
8. W. E. Parish, *Clin. Allergy,* **6,** 432-550 (1976).
9. G. D. Reynolds and R. P. Vance, *Arch. Pathol. Lab. Med.,* **111,** 265-269 (1987).
10. I. F. Rowe, A. K. Soutar, I. M. Trayner, *et al., Clin. Exp. Immunol.,* **58,** 237-244 (1984).
11. K. T. Sammalkorpi, V. V. Valtonen, and C. P. Maury, *Ann. Med.,* **22,** 397-401 (1990).
12. J. E. Volanakis and J. F. Kearney, *J. Exp. Med.,* **153,** 1604-1614 (1981).