MICROBIOLOGY AND IMMUNOLOGY

Reaction of C-Reactive Protein with Interleukin-2 Receptor α-Chain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 125, No. 6, pp. 657-660, June, 1998
Original article submitted April 23, 1997

Study of the mechanism of mutagenic effect of C-reactive protein, the major reactant of acute phase inflammation, showed that this protein is mitogenic for normal lymphocytes and inhibits proliferation of interleukin-2-dependent CTLL-2 cells in the presence of interleukin-2; antibodies to α-chain of interleukin-2 receptor abolish the inhibitory effect of C-reactive protein. High-affinity receptor of interleukin-2 may be assembled by interleukin-2 or C-reactive protein, but not by both ligands. Therefore, immunoregulatory effects of C-reactive protein are different during the acute phase of inflammation and remote periods of immune response. At the early stages, when the production of interleukin-2 is negligible, C-reactive protein can act as a mitogen inducing polyclonal activation of lymphocytes, while later it acts as a factor limiting clonal expansion of committed immunocompetent cells.

Key Words: *C-reactive protein; interleukin-2; interleukin-2 receptor*

C-reactive protein (CRP), an acute inflammation reactant and a factor of opsonization and clearance of bacterial and damaged cells, is a mitogen for human lymphocytes [2,3,5,13]. The mitogenic effect of CRP on lymphocytes is associated with its effect on the interleukin-2 (IL-2) receptor [1,8].

We showed that the IL-2 receptor is utilized by CRP in intact mouse lymphocytes and IL-2-dependent CTLL-2 cells, which exemplifies a versatile mechanism regulating the proliferation of cells sensitive to IL-2.

MATERIALS AND METHODS

Native human pentamer CRP (pCRP) and CRP monomers (mCRP) were prepared as described previously [2]. Human recombinant IL-2 (rIL-2, 10^7 U/mg) derived from *E. coli* (Institute of Organic Synthesis, Riga), monoclonal antibodies (MAb) to IL-2 receptor α-chain (ICO-105, anti-CD25) (Preparat, Nizhny Novgorod), human serum albumin (Reanal), and phosphate-buffered sterile normal saline, pH 7.2, were used. Mitogenic activity of pCRP and mCRP for mouse lymphocytes was assessed in 72-h cultures of male C57BL/6 mouse splenocytes (10^6 cells/ml) in RPMI-1640 (Flow) with 10% fetal calf serum (Institute of Vaccines and Sera, St. Petersburg) by ^{3}H-thymidine incorporation. CTLL-2 cells were cultured in RPMI-1640 with 10% fetal calf serum, L-glutamine, antibiotics, and the supernatant of concanavalin A-activated rat splenocytes as the growth factor. For assessing the capacity of CRP to maintain the growth of CTLL-2 without rIL-2, cells were transferred into fresh medium to which pCRP or mCRP were added in concentrations specified below. IL-2 was not added.

In the other series of experiments rIL-2 (12.5 U/ml) was added to CTLL-2 culture medium simultaneously with pCRP and mCRP (10 and 100 μg/ml,

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0007-4888/98/0006-0582$20.00

respectively) or human serum albumin (100 and 1000 μg/ml). MAb to CD25 in 1:2 dilution were added into the medium for blocking IL-2 receptor α-chains. The level of CTLL-2 proliferation was measured by ^{3}H-thymidine incorporation and compared with the standard curve plotted using the international human IL-2 reference specimen (No. 86/504). The data are presented as the number of pulses/min in percent of the control.

RESULTS

Comparison of mitogenic activity of CRP toward mouse cells of different origin showed that intact mouse splenocytes respond to pCRP by increased proliferation (Table 1), while IL-2-dependent CTLL-2 stopped proliferating in the presence of pCRP or mCRP if no rIL-2 was added (Fig. 1, *1, 2*). Thus, the effect of CRP on normal lymphocytes is not species-specific: CRP being as mitogenic for normal mouse lymphocytes as for human lymphocytes [2, 3,5,13]. On the other hand, CRP does not replace IL-2 as the growth factor for CTLL-2.

CTLL-2 proliferation did not change in the presence of rIL-2 after addition of the reference protein (human serum albumin, Fig. 1, *4*) but decreased by 90% after addition of pCRP or mCRP (Fig. 1, *5, 6*). Both conformations of CRP similarly abolish the proliferative response of human lymphocytes to rIL-2 without changing their response to phytohemagglutinin and concanavalin A [1,8]. Therefore, the inhibitory effect of pCRP and mCRP on IL-2-dependent proliferation of CTLL-2 cells is similar to that observed in cultured human lymphocytes.

The level of cell proliferation during incubation of CTLL-2 with rIL-2 and MAb to CD25 did not differ from that of cells cultured with rIL-2 without MAb (Fig 1, *3, 7*). The MAb are not blocking antibodies and their binding to the epitope does not prevent the receptor reaction with the ligand and generation of activated signal (Fig. 1). On the other hand, if CTLL-2 were incubated with MAb, IL-2, and CRP (in the p- or m-forms) simultaneously, blocking effect of pCRP and mCRP disappeared, and the cells reacted to rIL-2 similarly as in the control (Fig. 1, *8, 9*). This indicates a competition between MAb and CRP for the same site, the affinity of MAb for this site being higher than that of CRP.

A high-affinity IL-2 receptor consists of three chains [7,9]: 1) inducible α-chain (p55; CD25) with low affinity for IL-2; it does not conduct the signal but is necessary for the receptor complex formation; 2) permanently expressed β-chain (p75) binding IL-2 and transferring mitogenic signal to the cells; IL-15 reacts with it [4]; and 3) γ-chain (p65), significant for signal transfer but not affine for IL-2; it is present in the receptors for IL-4, -7, -9, and -15, where it is also responsible for signal transfer [12]. The formation of IL-2 receptor complex is initiated by the reaction of IL-2 with α-chain, which causes aggregation of other subunits, the dimerization of cytoplasmic domains of β- and γ-chains being obligatory for signal transduction [6].

CD25 antigen, to which the MAb are specific, is a marker of IL-2 α-chain; therefore, we can think that this chain contains an epitope reacting with both MAb and CRP. The consequences of α-chain binding to MAb and CRP are different. Activation signal is blocked during reaction of α-chain with CRP in the presence of rIL-2, which indicates the priority of CRP for binding to this chain and CRP claim for other chains, with which it can contact only after reaction with α-chain. When two ligands with different affinity for the receptor chains compete for the receptor assembly, deficient ineffective complexes may appear. In our case signal transduction could be impaired if CRP forms a complex with α- and γ-chains (not bound by IL-2), while IL-2 to β-chain alone. By activating intact lymphocytes in the absence of IL-2, CRP apparently utilizes a high-affinity IL-2 receptor consisting of α-, β-, and γ-chains.

When α-chain was bound to MAb, the mechanism of production of IL-2-dependent signal functioned normally; therefore, aggregation of receptor chains by these antibodies was not impaired. Moreover, the receptor assembled under the protection of MAb cannot be disordered by CRP, indicating that in the presence of antibodies the chains aggregate irreversibly and cannot be dissociated by CRP.

Clinical findings indirectly point to the capacity of CRP to modulate the response to IL-2. In cancer patients treated with rIL-2 favorable clinical effect was observed only when blood CRP level slightly increased during IL-2 infusions (no higher than 10 mg/liter), while patients with a greater increase in CRP levels did not respond to therapy with IL-2 [11].

The production of IL-2 is negligible during the acute phase of inflammation. Presumably, at this

Table 1. Mitogenicity of Pentamer CRP for Intact Mouse Splenocytes (*M±m*)

pCRP concentration in medium, μg/ml	^{3}H-thymidine incorporation, pulse/min	Stimulation index
100	3385±245	10.4
25	1108±110	3.4
0 (normal saline)	326±33	

Note. *Determined for 4 cultures containing splenocyte pools from 4 mice.

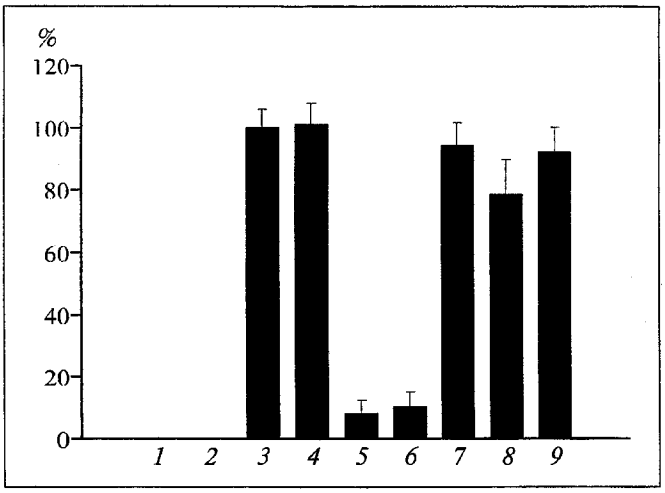

Fig. 1. Proliferation of CTLL-2 in the presence of recombinant interleukin-2 (rIL-2), pentamer (pCRP) and monomer (mCRP) C-reactive protein (in percent of response to IL-2). *1, 2*) CTLL-2 proliferation in the presence of pCRP and mCRP (10 μg/ml) without rIL-2; *3*) CTLL-2 proliferation in the presence of rIL-2 (12.5 U/ml); *4*) rIL-2+human serum albumin (100 μg/ml); *5*) rIL-2+pCRP (10 μg/ml); *6*) rIL-2+mCRP (10 μg/ml); *7*) rIL-2+monoclonal antibodies (MAb); *8*) rIL-2+pCRP (10 μg/ml)+MAb; *9*) rIL-2+mCRP (10 μg/ml)+MAb.

early stage of defense, before induction of antigen-specific T-cells, CRP compensates for IL-2 deficit by ensuring mitogenesis and recruiting lymphocytes. Expression of α-chain on native lymphocytes is probably activated during this process. At least, the expression of CRP coincides with the appearance of IL-2 "soluble receptor" (α-chain free extracellular domain) in the circulation, though their blood levels do not strictly correlate [10]. The biological role of free α-chain is not clear, but the duet of free α-chain and CRP during this period may stimulate the efficacy of minor amount of IL-2: α-chain protects IL-2 from proteases, while CRP ensures dissociation of the complex and transfers IL-2 to sensitive cells. This problem deserves special studies. At later periods, when the production of IL-2 by T-helpers notably increases, CRP can participate in limiting further expansion of immunocompetent cells by blocking IL-2-dependent mitogenic signal described above.

The author is grateful to Dr. A. Cimanis (Institute of Organic Synthesis, Riga) and to Dr. L. K. Berestovaya (Institute of Vaccines and Sera, St. Petersburg) for recombinant IL-2 and CRP, and to Drs. N. V. Pigareva and A. S. Simbirtsev, (Institute of Highey Pure Biological Preparations, St. Petersburg) for assistance in experiments of CTLL-2 cells.

REFERENCES

1. P. G. Nazarov and A. V. Polevshchikov, *Vestn. Rossiisk. Akad. Med. Nauk.*, No. 9, 13-17 (1993).
2. P. G. Nazarov, A. V. Polevshchikov, L. K. Berestovaya, *et al., Byull. Eksp. Biol. Med.*, **116,** No. 12, 609-611 (1993).
3. P. G. Nazarov, A. V. Polevshchikov, and L. K. Berestovaya, *Immunologiya,* No. 4, 15-17 (1994).
4. J. G. Giri, M. Ahdieh, J. Eisenman, *et al., EMBO J.,* **30,** 2822-2825 (1994).
5. M. Hornung and S. Fritchi, *Nature: New Biol.,* **230,** 84-85 (1971).
6. T. Kishimoto, T. Tanaka, K. Yoshida, *et al., Ann. N. Y. Acad. Sci.,* **766,** 224-234 (1995).
7. T. Nakamura, S. M. Russel, S. A. Mess, *et al., Nature,* **369,** 330-333 (1994).
8. P. G. Nazarov and A. V. Polevschikov, *Lymphokine Res.,* **12,** No. 5, 400 (1993).
9. N. Nicola, *Ann. N. Y. Acad. Sci.,* **766,** 253-262 (1995).
10. A. Sheldon, M. J. Ahern, M. D. Smith, *et al., Clin. Exp. Rheumatol.,* **12,** No. 2, 175-178 (1994).
11. W. G. Simpson, S. D. Heys,P. H. Whiting, *et al., Clin. Exp. Immunol.,* **99,** No. 2, 143-147 (1995).
12. T. Taniguchi, T. Miyazaki, Y. Munami, *et al., Ann. N. Y. Acad. Sci.,* **766,** 235-244 (1995).
13. M. L. Vetter, H. Hewurz, B. Hansen, *et al., J. Immunol.,* **130,** No. 5, 2121-2126 (1983).