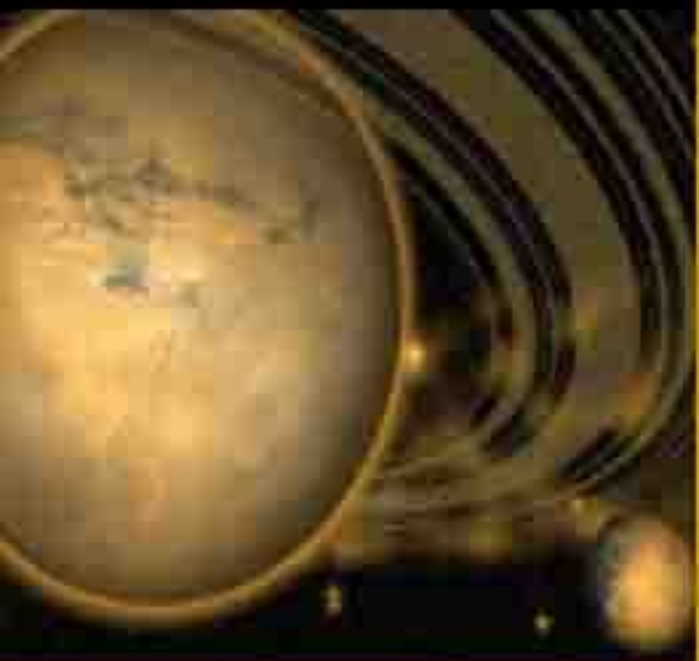


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C-reactive protein: Fc-gamma receptor-mediated effects on human peripheral blood basophils in vitro.

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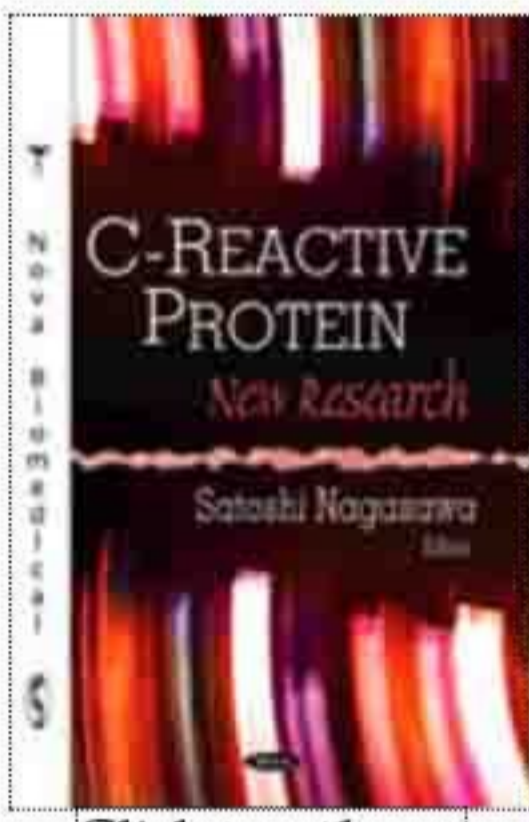
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C-reactive protein: Fcγ receptor-mediated effects on human peripheral
blood basophils in vitro

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Introduction

Described by Paul Ehrlich in 1879, basophilic granulocytes (basophils) remain among the least studied white blood cells. A rather small number of works on them (compared to other leukocyte types) was attributable to their extremely low content in the blood (0,5-1% of nucleated blood cells), but not to lack of interest by scientists. The number and morphology of basophils are somewhat different in different animals, but they occur in all classes of vertebrates. It demonstrates the importance of these cells for protective systems. Basophils are involved in allergic reactions and anti-parasitic protection. Moreover, due to cytokine secretion, in particular IL-4 and IL-13, they became the focus of attention as a cell type implicated in the immune response switch toward Th2 [Falcone et al., 2006]. The relationship of basophils with the acute phase of inflammation factors, in particular, with C-reactive protein (CRP), are poorly elucidated.

Elevated expression of CRP, a pentraxin and known marker of acute phase of inflammation, accompanies the onset of any form of immune response. However, CRP influence on the intensity and specificity of immune reactions is not clearly understood. It has been known that a good correlation exists between CRP production at early stage of immunization and subsequent antibody production in rabbits. It has been also shown that CRP could influence the fine specificity of immune responses to antigens which are its ligands. CRP could decrease protective antibody production against pneumococcal cell wall phosphorylcholine (PC). PC binding by CRP leads to the opsonization of pneumococci, complement and phagocytosis activation [Nakayama S. et al., 1984; Horowitz J. et al., 1987; Szalai A.J. et al., 1995]. There are numerous data concerning stimulating effects of CRP on phagocytic activity and other functions of macrophages and neutrophils. CRP has been shown to depend for its biological function on cellular Fc γ receptors (Fc γ Rs). Fc γ RI is a high-affinity IgG receptor expressed on myeloid cells that is up-regulated during inflammation [Van de Winkel J.G.J., Anderson, C.L., 1991; Van de Winkel J.G.J., Capel P.J., 1993; Beekman J.M. et al., 2004]. However, the major receptor for CRP on leukocytes is low affinity Fc γ RII [Bharadwaj D. et al., 1999].

The least understood is the character of the pentraxin influence upon the immediate type hypersensitivity reactions. There are few data suggesting suppressive effects of CRP on allergy development. Thus, it has been shown that the levels of CRP and IgE were negatively correlated in the population of asbestos workers [Lange A. et al., 1995]. However these data do not answer all the questions concerning participation of pentraxins and especially CRP in allergic reactions of acquired immunity.

We have shown in guinea pigs with experimental anaphylaxis that the injection of purified CRP prior to antigen attenuated anaphylactic reaction to a significantly greater extent than the injection of normal human IgG [Nezhinskaya G.I., Nazarov P.G., Evdokimova N.R., Losev N.A., Sapronov N.S., 2004; Nazarov P.G. et al., 2005]. We have also shown that purified human CRP diminished effects of acetylcholine (ACh) on the vascular tone and the heart rate of rats in vivo. In vitro CRP inhibited breakdown of ACh by acetylcholinesterase (AChE) while did not interact with AChE itself. CRP did not modify the cardiovascular effects of adenosine, another vasorelaxant. These data suggest that ACh sharing structural features with phosphorylcholine, the classical ligand of CRP, can also be a ligand of the pentraxin [Nazarov P.G. et al., 2006, 2007]. CRP might act on ACh itself by capturing it or on cellular ACh receptors by blocking them or modifying their function. Ameliorating effect of CRP on the development of allergic sensitization and anaphylactic shock, we have described, could be due to the fact that CRP binds ACh and reduces its proinflammatory effects. Moreover, anti-anaphylactic effect of CRP could also be linked to the action of the pentraxin on basophils and mast cells, but this question is not good elucidated in the literature.

Blood levels of CRP are positively correlated with cardiovascular disease risk and endothelial dysfunction. An inhibitory effect of CRP on endothelial function has also been shown by other authors who explained it by the decrease in NO production due to inhibition of PP2A phosphatase and down-regulation of endothelial NO synthase [Devaraj S. et al., 2005; Liang Y.-J. et al., 2006]. CRP antagonism of eNOS was reported to be attributable to blunted eNOS phosphorylation at Ser1179. This was supported by the data that in the absence of active PP2A (due to inactivation by short-interference RNA) CRP had no effect on eNOS.

Our previous data are inconsistent with the conclusion that the eNOS down-regulation and NO production impairment by CRP are major reasons of endothelial dysfunction caused by the pentraxin. Our data have indicated that CRP had no effect on vascular relaxation caused by adenosine, the known NO inducer [Nazarov P.G. et al., 2007]. However, whether CRP has direct actions on endothelial cells and the mechanisms underlying such actions are unknown.

One of the mechanisms of the anti-anaphylactogenic effect of CRP shown by us earlier could be associated with the pentraxin inhibitory action on tissue mast cells and blood basophils responsible for the release of vasoactive mediators and the response of vessels and bronchi in allergic reactions of immediate type. Mast cells and basophils are activated by IgE antibodies and relevant antigen through IgE receptors (FceRI). The receptors FcgRI and FcgRIII, binding IgG subclasses (IgG1, IgG2, IgG3), are also activators of these cells, whereas FcgRIIb, on the contrary, inhibits cell activity and cancels activation signals of other receptors. Such pentraxins as CRP and serum amyloid P-component (SAP) known to be actively expressed in acute phase

of inflammations influence the immunocompetent cell activity also through Fc-gamma R. The role of pentraxins in activation of mast cells and basophils is poorly defined. The cholinergic regulation of mast cell and basophil activity is also poorly understood, in particular in view of recent attention to autonomous, non-neuronal cholinergic system of immunocompetent cells which includes such elements as ACh synthesis, ACh destruction, and ACh receptors of muscarinic and nicotinic type (mAChR and nAChR) [Kawashima K., Fujii, T., 2000; Kirkpatrick, C.J. et al., 2003; Wessler I. et al., 2003]

The aim of the study was to investigate the ability of C-reactive protein to activate human blood basophils *in vitro* and to elucidate the role of autonomic cholinergic system elements (ACh and AChRs) in modulation of the pentraxin effect on basophils. Basophil functional activity activated by CRP or other inducers was investigated in the presence or absence of cholinergic blockers, such as mAChR and nAChR antagonists.

Materials and Methods

Basophils isolation

Basophils were isolated from heparinized peripheral blood of healthy donors. For each separation approximately 30-40 ml of venous blood from one donor were used. The first step of the protocol was the preparation of leukocyte mixture by spontaneous red blood cells sedimentation (approximately 30 min at 37 °C) or by gradient centrifugation in Ficoll-Paque. Human Basophil Isolation Kits II purchased from Miltenyi Biotec was used for basophil separation. Separation protocol was based on negative selection of basophils. All leukocytes except basophils were first treated with a cocktail of biotinylated monoclonal antibodies against CD3, CD4, CD7, CD14, CD15, CD16, CD36, CD45RA, HLA-DR and CD235a and then with anti-biotin monoclonal antibody conjugated with magnetic microbeads. Magnetic particles with attached cells were then removed using MACS® Column and magnetic field in MACS Separator. This approach provided a highly enriched basophil fraction. The cells eluted from the column were qualified as intact because they did not contact with any antibody.

Isolated basophils were analysed by flow cytometry on Epics Altra Cell Sorter (Beckman-Coulter) using FITC-labelled monoclonal antibodies against CD63 and CD203c. CD63 (gp53, or lysozyme-associated membrane protein, LAMP-3) is a transmembrane protein expressed not only by basophils, but also by tissue mast cells, macrophages and platelets [Nieuwenhuis H.K. et al., 1987; Metzelaar M.J. et al., 1991; Grutzkau A. et al., 2004]. In resting basophils and mast cells CD63 is hidden inside the cell being a component of cytoplasmic

granules, but after activation appears on the cell surface. On the IgE-activated basophils, during their degranulation, CD63 is expressed in high density due to fusion of the granules with cell surface membrane [Knol E.F., 1991]. CD203c (neural cell surface differentiation antigen E-NPP3; PD-Ib, B10, gp130RB13-6) belongs to a multigene family of ectonucleotidetriphosphatases/phosphodiesterases (E-NPPs), that includes also E-NPP1 (PC-1, PDNP-1) and E-NPP2 (PD-Ia, PDNP2, autotoxin). In peripheral blood, CD203c is expressed exclusively on the surface of basophils [Bühning H.J. et al., 2004]. In contrast to CD63, CD203c is expressed on the surface of resting basophils and after their activation the expression of CD203c is only slightly enhanced; that's why this marker is less prominent than CD63.

Reagents

Armine, an irreversible AChE inhibitor, was used as 0.01% solution. Final concentration in cell suspension was 2 µg/ml or 0.2 µg/ml. Hexamethonium, a nicotinic AChR antagonist, was used as a sterile 2.5% solution for injections; final concentration was 4.16 mg/ml. Methacine (sterile 0.01% solution for injections); final concentrations were 0.017% (0.17 mg/ml) or 0.0017% (0.017 mg/ml). Armine, hexamethonium and methacine were purchased from Kazan Pharmaceuticals (Russia), concanavalin A (ConA) from Pharmacia Fine Chemicals (Sweden), compound 48/80, carbachol (carbamylcholine chloride) and histamine dihydrochloride and – from Sigma-Aldrich. Concentrations of carbachol and ConA are indicated under “Results and Discussion” section.

Human C-reactive protein (CRP) was obtained from MB-Biochemicals, human serum amyloid P-component (SAP) – from Calbiochem, histamine diphosphate salt – from Sigma-Aldrich, normal human immunoglobulin (IgG) – from Institute Pasteur (St. Petersburg, Russia), anti-CD16 monoclonal antibody (ICO-116) – from MedBioSpektr (Moscow, Russia). Phosphate buffered salt solution (PBS) contained 0.14 M sodium chloride in bidistilled water buffered with a mixture of phosphate salts, pH 7.2. Heat aggregated IgG (aIgG) was prepared by heating IgG solution at a concentration of 10 mg/ml in PBS in a water bath at 63°C for 10 min. Prior to use, solutions of commercially available CRP and SAP were dialyzed overnight at 4°C against large excess of sterile PBS using Slide-A-Lyzer Cassettes (Pierce) to remove NaN₃. LPS of *Salmonella typhi* was purchased from the Institute of Vaccines and Sera (St. Petersburg, Russia).

Cell incubations and assessment of basophil response

Isolated and washed blood cells were suspended in PBS and put into eppendorf polypropylene tubes, 10⁵ cells per tube in 800 µl on ice. CRP (or aIgG, or SAP) was added in appropriate volume to get final concentrations indicated under the “Result and Discussion” section. Cholinergic reagents (nAChR or mAChR antagonists, AChE inhibitor, or PBS for control tubes) were added immediately after. Total volume was 960 µl, including cells, a protein stimulant, and a cholinergic agent. Tubes were placed into

incubator and incubated at 37°C for 30 min. Then the tubes were cooled on ice and centrifuged at 400 g for 10 min at 4°C. Supernatants were collected and frozen at -20°C for histamine determination. Cell pellets were frozen separately for total histamine content determination. The Shore method with orthohtalic aldehyde and fluorometric detection [Fujimoto T. et al., 2003] was used. Triplicates of each sample were measured in a microplate fluorometer (Victor 5, Wallac) at wavelengths of 340 (excitation) and 450 nm (emission). The numbers of experiments are as indicated in Figures below.

Concentration of histamine in the supernatants was estimated from the fluorometer readings using a linear calibration plot built in each experiment with standard histamine solutions. The data on histamine secretion are presented as nanograms of histamine recovered in cell supernatants after incubation with stimulants (ng/ml per 10^5 cells), as well as stimulation indexes which were calculated as ratio of histamine (in ng/ml) released in the presence of the agents to spontaneous histamine release (ng/ml) in PBS (the latter was taken to be 1.0).

Basophil desensitization and restimulation in vitro

Leukocyte fraction isolated from fresh healthy donor blood by spontaneous erythrocyte sedimentation was used in these experiments. The cells were suspended in RPMI 1640 tissue culture medium (Biolot, St. Petersburg, Russia) supplemented with 10% fetal calf serum (FCS, Flow Laboratories, UK) of L-glutamine (2 mmol/L) and gentamicin sulfate (50 mg/ml) and cultivated in 24-well plastic plates (Linbro) (5×10^5 per well) in CO₂-incubator (Flow) at 37°C for 48 hr. The stimulations of the cultures were performed 2 or 3 times, once a day. The first stimulation was at the beginning of the cultivation. For this purpose, CRP, aIgG, ConA, Cch, or PBS were added to the cultures for 40 min at 37°C. Thereafter the cells were sedimented, supernatants removed for histamine determination, and the cell pellets washed thrice with PBS, resuspended in fresh tissue culture medium (its composition see above) and allowed to cultivate further at 37°C. Washing fluids after last wash were analyzed for histamine content to control the completeness of washing. On the next day, the cell cultures were restimulated with the same stimulants by adding them again to the culture medium for 40 min at 37°C. Histamine concentration in test supernatants and control washing fluids was determine by Shore method.

Statistical analysis

Values presented in Figures are means (M) \pm standard errors of mean (SEM). Student *t* test was used to assess differences between groups, and significance was set at $p < 0.05$. Stimulation indices were calculated as ratio of histamine quantity (in ng/ml) released in the presence or each agent to spontaneous histamine release (ng/ml) in PBS (taken to be 1.0).

Results and Discussion

1. Basophil responses to human aggregated IgG, C-reactive protein, and FcγRIII cross-linking

Aggregated IgG is a well-known ligand for Fcγ receptors. The data on histamine release from healthy donor blood basophils incubated in vitro with human aIgG, CRP, SAP and monoclonal anti-CD16 antibody are shown in Figure 1. In our experiments, both normal human IgG and normal human IgG aggregated by heating at 63 C caused significant increase in histamine release. Heat aggregated IgG was slightly more active in basophil stimulating compared to non-aggregated IgG preparation. The mean stimulation index for aIgG (100 μg/ml) was 8.5 ± 1.2 (n=3) whereas for non-aggregated 5.6 ± 1.1 (n=3). Difference between the effects of IgG and aIgG was not significant ($p > 0.05$).

Human CRP induced a dose-dependent basophil responses with mean stimulation indexes being 0.94 ± 0.02 (n=3) at 10 μg/ml, 3.56 ± 0.06 (n=6) at 50 μg/ml, and 1.67 ± 0.02 (n=3) at 100 μg/ml. So CRP enhanced histamine secretion in a dose-dependent way with a maximum at 50 μg/ml. SAP caused a weak stimulation of basophil secretion (mean stimulation index with SAP was 1.215 ± 0.02 (n=3), whereas anti-CD16 antibody caused apparent stimulatory effect ($p < 0.05$). These data indicate that cross-linking of low affinity FcγRIII activates basophils to enhance histamine release.

Our experimental data show (Figure 1) that the effect of human pentraxin CRP upon histamine liberation by basophils was similar to the effects of other agents capable of binding to FcγR, such as aggregated IgG or anti-FcγRIII monoclonal antibody. All of them enhanced histamine liberation from basophils.

A moderate but statistically significant histamine release was stimulated by incubation with *S. typhi* LPS (25 μg/ml) (Figure 1). The most effective histamine liberator was the compound 48/80 stimulated the mean histamine release at a level of about 73% of the total histamine content in the cells (Figure 1).

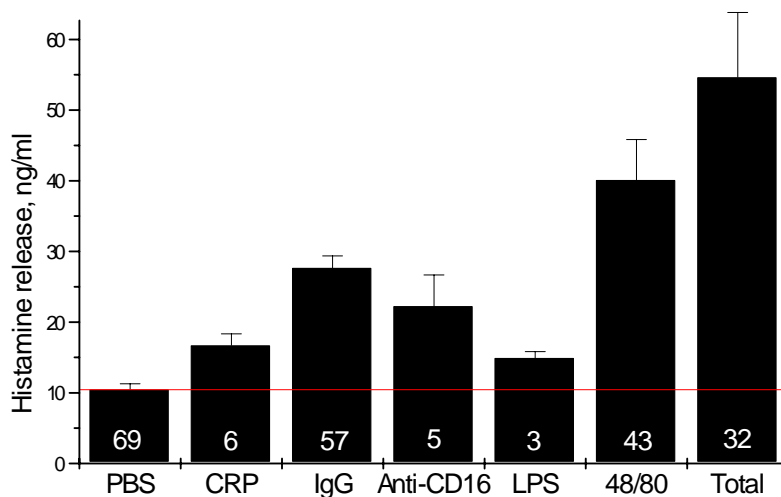


Figure 1. Histamine release from normal human blood basophils incubated *in vitro* with human IgG, CRP, and anti-CD16 antibody.

Numbers at the bottom of the columns represent the number of experiments.

2. Basophil responses to cholinergic drugs

A list of used cholinergic drugs included: carbachol, a non-metabolizable chemically related analog of ACh that does not readily enter cells; hexamethonium, a nicotinic ACh receptor antagonist; methacine, a muscarinic AChR antagonist; and armine, an irreversible inhibitor of AChE.

Carbachol displayed a two-zone effect depending on its concentration in the medium. Its dose-response curve is shown on Figure 2. At high doses (0.15-1.5 mg/ml) carbachol was suppressive and inhibited histamine response below the level of spontaneous release observed with PBS. At a lower concentration it showed stimulatory effect on basophils and elevated histamine secretion twofold compared to control level. The carbachol dose of 15 μ g/ml was selected as the optimal for further experiments.

Figure 3 shows the effects of four cholinergic drugs on histamine release from human basophils *in vitro*.

Of interest is the effect of armine on basophil secretion. Under its action a complete block of AChE occurs leading to complete termination of ACh breakdown and accumulation of undestroyed, active ACh. So, in the presence of armine the excessive formation of ACh inside the cells and in their environment should occur. As can be seen from Figure 3, the increase in

basophil histamine secretion in the presence of armine did occur, like that observed under the direct action of ACh-related carbachol ($p>0.05$). We have tested two concentrations of armine, 0.2 and 2 $\mu\text{g/ml}$. Figure 3 shows the effect of only one of them (2 $\mu\text{g/ml}$); data on the effect of 0.2 $\mu\text{g/ml}$ are not shown. Difference between the effects of these two doses of armine was not statistically significant ($p>0.05$).

It can also be seen from Figure 3 that the blockade of nicotinic AChRs with hexamethonium at a final dose of 4.17 mg/ml resulted in a 1.5-fold stimulation of histamine secretion. Hexamethonium stimulatory effect was reproducible and statistically significant ($p<0.05$). Hexamethonium is largely a nAChR blocker and is supposed to occupy only the nicotinic part of the cell AChR pool. Its enhancing effect on basophil activity might be due to a compensatory increase in cholinergic signaling through an antagonistic pathway associated with mAChRs.

Similar stimulating effects were seen with methacine. This muscarinic antagonist produced an enhancement of histamine secretion in low dose of 0.017 mg/ml (Figure 3) while the higher dose of 0.17 mg/ml showed no effect on basophil secretion (Figure 3). This result may be attributable to the reasons mentioned above for hexamethonium. Occupation of muscarinic subpopulation of cellular AChRs by methacine could facilitate a compensatory ACh flow via alternative nAChRs.

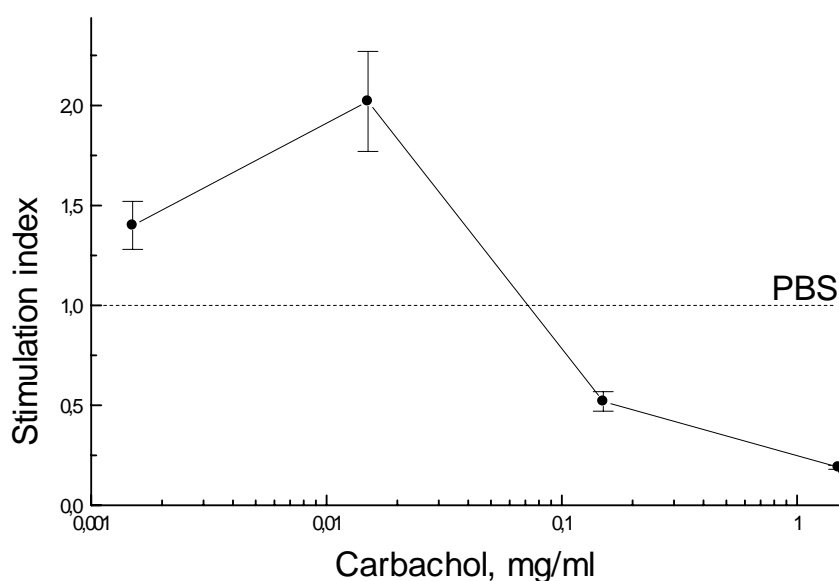


Figure 2. Human blood basophil responses to carbachol. A dose – response curve. Each point is a mean of three tests \pm SEM.

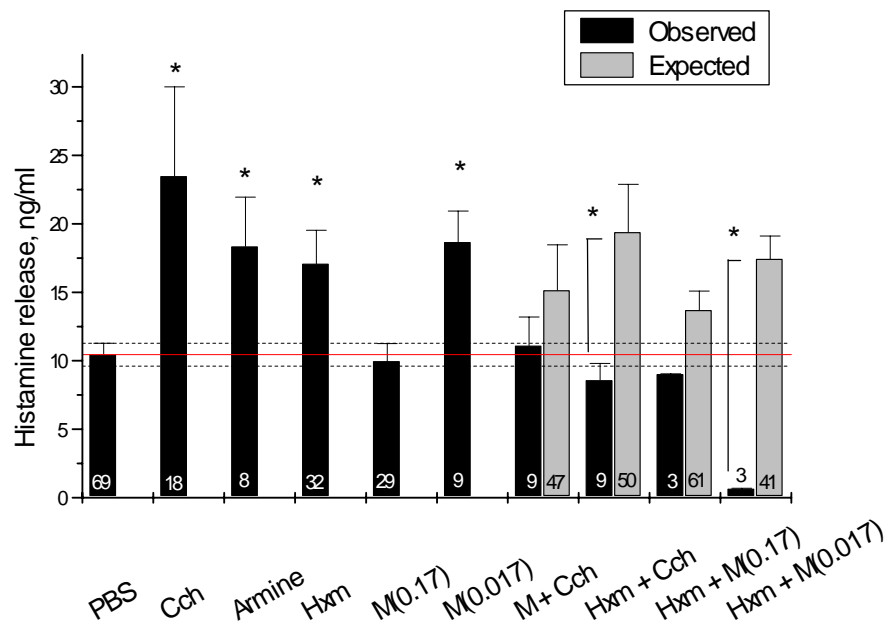


Figure 3. Human blood basophil responses to hexamethonium, methacine, armine and their combinations.

Numbers at the bottom of the columns represent the number of experiments.

Thus, carbachol and armine stimulated histamine secretion by normal human blood basophils. Carbachol is known to be similar to ACh by its activity, while differs from it by the resistance to AChE action. Based on the effect of carbachol on basophils one can get idea of the effect of ACh. Armine blocks the AChE and stops endogenous ACh destruction, thus increasing the influence of ACh on cells. So the increase in histamine secretion induced by either carbachol or armine may be attributable to ACh activity. Therefore, it can be concluded that the effects of carbachol and armine are associated with ACh activity. Conceivably both carbachol and armine might mediate their effects through either nAChR- or mAChR-dependent pathways.

By combined application of carbachol with nAChR or mAChR antagonist we have analyzed the question of which type of AChRs mediated the action of exogenic carbachol and endogenous ACh accumulating under the AChE inhibition. As to carbachol, some data suggest that it is a selective agonist of muscarinic AChRs [Oishi K. et al., 2004]. In contrast to these data, however, another evidence exists indicating the absence of selectivity of carbachol [Xiao Y. and Kellar K.J., 2004]. In addition, we could not find published data on the nAChR or mAChR selectivity of endogenous ACh accumulating under the AChE inhibition.

Statistical comparisons of the observed combined effects of the drugs were made with the expected (rated) values calculated from their separate effects. Figure 3 shows separate effects of drugs, their real combined effects, and the calculated values of combined effects referred to as “expected”, or “rated”.

As can be seen, incubation of the cells with carbachol together with methacine induced much lower histamine release than expected. Similarly, combination of carbachol with hexamethonium also provided significantly less pronounced effect of histamine release than could be expected based on their separate effects ($p < 0.05$, Figure 3). The use of calculated (“expected”) values rather than real separate controls seemed to be more correct for comparison purposes since such calculated values would represent more weighted average estimates of any joint effect than do any of the source control values. So, the comparisons with the “expected” effects indicated that the blockade of either nAChRs or mAChRs resulted in cessation of stimulatory effect of carbachol on histamine release from blood basophils in vitro. The inhibitory effect of nAChR antagonist hexamethonium was even more pronounced than the effect of muscarinic antagonist methacine. These data do not fit the concept that carbachol has muscarinic selectivity. Our results suggest that this ACh analog can provide its activation signals both to nAChRs and mAChRs.

The last point from Figure 3 to be discussed is the combined effect of two AChR antagonists. In these experiments hexamethonium was used in one dose (4.17 mg/ml), while methacine in two (0.017 and 0.17 mg/ml). Both hexamethonium and methacine when acting singly enhanced basophil histamine secretion (Figure 3). Treatment of the cells with their mixture led to the cessation or even abrogation of stimulating effect. It suggests that the withdrawn of any cholinergic pathway (either muscarinic or nicotinic) results in compensatory enhancement of signaling through another one. The blockade of both AChR types cuts off signaling, and ACh can not further activate the cells. As can be seen from Figure 3, a combination of hexamethonium with methacine was less stimulatory than expected. The most suppressive was the lower dose of methacine (0.017 mg/ml). In combination with hexamethonium it suppressed basophil activity significantly, much below background level ($p < 0.05$). The reason of such profound suppression may be that the low dose of methacine selects and triggers a high affinity subset of mAChRs that has a suppressive function in basophil physiology control, and this results in the activation of a suppressive mechanism like heterologous desensitization.

3. Basophil responses to aggregated IgG and cholinergic drugs

Aggregated IgG was used as the classical ligand for FcγRs. As can be seen from Figure 4, aIgG was able to induce histamine secretion by normal blood basophils from healthy donors. At a concentration of 100 μg/ml aIgG stimulated a 2.5-fold increase in histamine release that was statistically significant compared to spontaneous level ($p < 0.05$). This effect is believed to be a result of FcγR cross-linking on the basophil cell surface membrane.

Addition to the cells of the nicotinic antagonist hexamethonium together with aIgG led to a decrease in the induced histamine release compared to both aIgG effect and “expected” effect calculated for combined IgG+Hex action (Figure 4, $p < 0.05$). Expected estimate was calculated using corresponding values of separate effects of aIgG and hexamethonium (for these separate values, see Figure 3). As nicotinic antagonist hexamethonium significantly decreased the aggregated IgG basophil response, it can be concluded that autonomic cholinergic system of basophils, particularly nAChRs, takes part in the mechanisms of basophil degranulation and histamine secretion induced by aIgG through FcγRs. Nicotinic AChRs seem to play a costimulatory role in this process.

Moreover, the addition of muscarinic antagonist (at a dose of 0.17 mg/ml) to the above mixture of aIgG and hexamethonium resulted in further suppression of basophil response. Histamine release dropped to the background level. This effect of methacine was statistically significant compared to both aIgG effect and “expected” effect of IgG+Hex+Meth combination (Figure 4).

The effects of hexamethonium and methacine indicate that ACh signaling via both nicotinic and muscarinic AChRs are needed for and participate in the IgG stimulating effect on basophil histamine secretion. Cholinergic signaling seems to be evoked during FcγR cross-linking by IgG aggregates. It can include FcγR-activated enhancement of ACh synthesis with further autocrine self-stimulation of the cells that produced it or/and paracrine affecting the nearby cells.

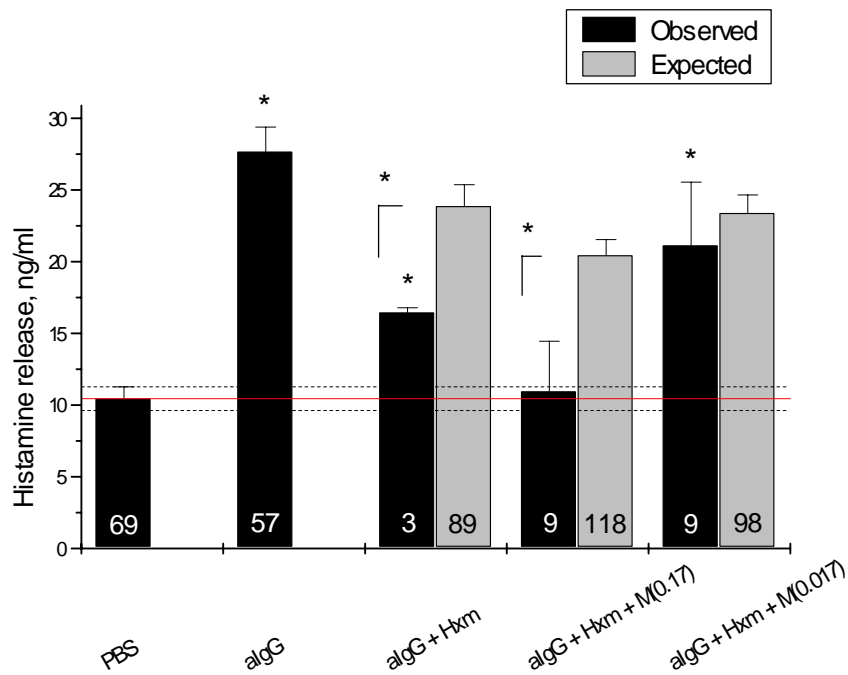


Figure 4. Human blood basophil responses to aggregated IgG. Effect of nAChR and mAChR blockade.

IgG – heat aggregated normal human IgG, 100 μ g/ml; Hxm – hexamethonium, 4.16 mg/m; M(0.17) and M(0.017) – methacine, 0.17 and 0.017 mg/ml, respectively. Expected values were calculated using effect of aIgG and separate effects of Hxm, M(0.17) and M(0.017) that are shown on Figure 3. Numbers at the bottom of the columns represent the number of experiments.

The addition of a lower dose of muscarinic antagonist (0.017 mg/ml) to the aIgG plus hexamethonium mixture resulted in the cancellation of hexamethonium inhibitory effect. Really observed mean level of histamine release under the action of the aIgG+Hxm+Met(0,017) mixture does not differ not only from the calculated expected value but also from the observed effect of aIgG alone. This evidence supports the above suggestion that a low-dose effect of muscarinic antagonist methacine may be based on the inhibitory function of high affinity mAChRs occupied by small quantity of the drug molecules.

The results of this section show that aggregated IgG when affecting the cells involves more than one type of receptors. In addition to Fc γ Rs which it binds and cross-links as their ligand it may concomitantly affect other receptor systems. One of such foreign receptor system that is obviously involved in the aIgG basophil response is cholinergic pair of two AChR types, nicotinic and muscarinic.

4. Basophil responses to human C-reactive protein and cholinergic drugs

As can be seen from figures 1 and 5, CRP activates histamine release from human blood basophils. After incubation of basophils with the combinations of CRP plus carbachol or CRP plus armine the significant inhibition of histamine release was registered compared with either the observed effect of CRP alone or with the calculated estimates of expected effects of the two agents of which each has demonstrated basophil activating activity. Instead of high levels of histamine release that we might expect based on the separate effects of CRP, carbachol and armine, we observe significantly lower histamine release in both cases. Really observed effects of CRP+Cch and CRP+armine significantly differed from both expected estimates (Figure 5, $p < 0.05$ for both comparisons). This makes it possible to reject null hypothesis that there was no cooperation between CRP and carbachol as well as between CRP and armine. The data suggest that some kind of CRP interaction with these cholinergic agents does take place. As we have previously shown [Nazarov P.G. et al., 2007], CRP binds acetylcholine and masks its effects because of structural similarity between ACh and phosphocholine (PC, the major ligand of CRP).

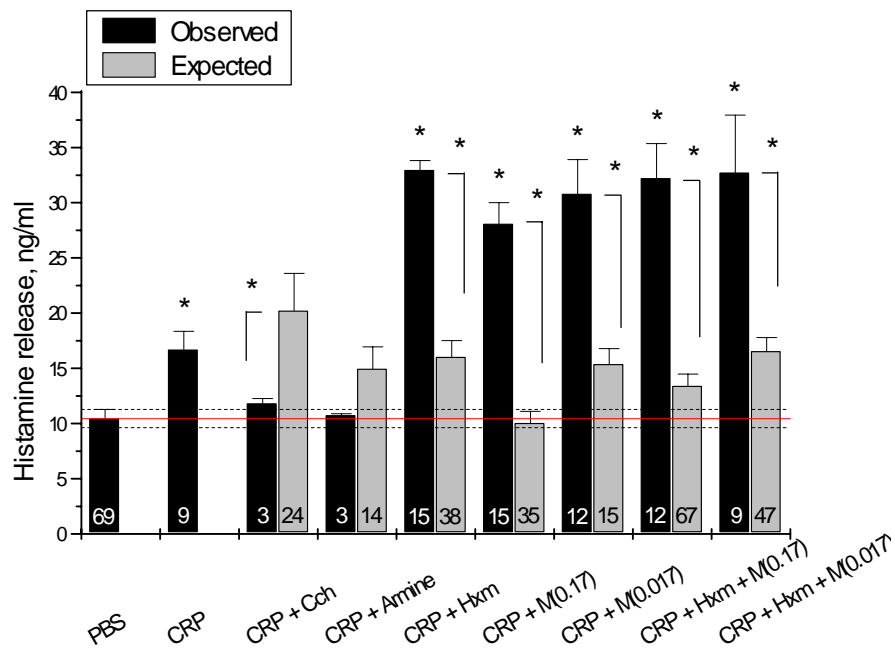


Figure 5. Human blood basophil responses to CRP: effect of nAChR and mAChR blockade.

CRP – human CRP, 50 $\mu\text{g/ml}$; Cch – carbachol, $\mu\text{g/ml}$; armine – irreversible AChE inhibitor, 2 $\mu\text{g/ml}$; Hxm – hexamethonium, 4.16 mg/m ; M(0.17) and M(0.017) – methacine, 0.17 and 0.017 mg/ml , respectively. Separate effects of Hxm, armine, M(0.17) and M(0.017) that

were used in calculation of expected estimates plotted here are shown on Figure 3. Numbers at the bottom of the columns represent the number of experiments.

The carbachol molecule is closely related to both Ach and PC molecules (Figure 6), which allows to assume that carbachol binding by CRP might occur. As to armine, the resulting product responsible for its biological activity is Ach as well. So, the results shown on Figure 5 suggest that low basophil responses to CRP combinations with carbachol and armine were associated with a decrease in local effective concentration of endogenous Ach (in the case of armine) or carbachol due to their binding by CRP rather than with an active mechanism of suppression.

Incubation of normal basophils with CRP combined with AChR antagonists, hexamethonium or methacine, resulted in significant enhancement of CRP histamine release-inducing activity. The levels of secreted histamine after stimulation of the cells with CRP+Hxm or CRP+M (with either dose of metacine, 0.17 or 0.017 $\mu\text{g/ml}$) were much higher than observed with CRP alone ($p < 0.05$) or expected for CRP combination with these cholinergic antagonists ($p < 0.05$ for any combination) (Figure 5).

In contrast to expected moderate decrease of CRP-induced basophil secretion in the presence of nAChR and mAChR blockers, both cholinergic antagonists enhanced basophil degranulation and histamine release. This situation is quite different from that showed above for aggregated IgG and its combinations with cholinergic blockers. Rather than lower the basophil reaction to CRP like observed with aIgG, AChR antagonists highly elevated it. It suggests that CRP can affect biological activity of hexamethonium and methacine by unknown mechanism.

We have reported earlier that CRP diminished the severity of the anaphylaxis reaction when applied either in the period of animal primary sensitization with a protein antigen or 30 min before the injection of a resolution dose of antigen to sensitized animals [Nezhinskaya G.I. et al., 2004, 2005]. Methacine in these experiments significantly reduced the severity of the anaphylactic shock. However, if methacine was injected together with CRP immediately before the resolution injection of the antigen, there was no decline in the intensity of the shock, which should be expected since methacine and CRP caused protective effect separately. Instead a significant increase in the severity of shock was observed [Nezhinskaya G.I. et al., 2004, 2005]. This observation indicated that CRP and methacine could interact and neutralize each other.

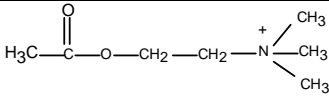
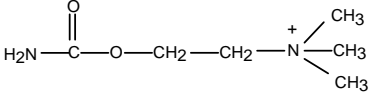
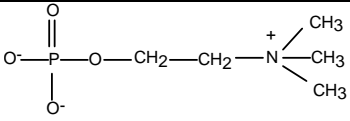
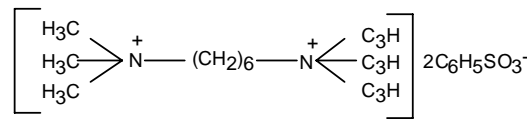
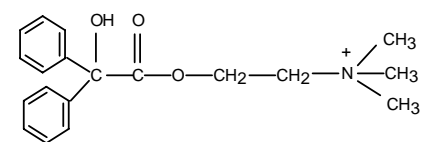
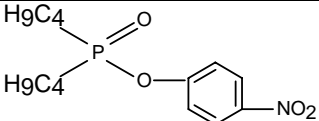
	Acetylcholine
	Carbachol
	Phosphocholine
	Hexamethonium (1,6-bis-(N-trimethylammonium)-hexane dibenzolsuphonate)
	Methacine (beta-dimethylaminoethyl ether of benzyl acid, iodine methylate)
	Armine (ethyl-p-nitrophenyl ether of ethylphosphonic acid)

Figure 6. The structure of cholinergic compounds used in the study.

The development of acute shock in those experiments was significantly accelerated by muscarinic acetylcholine receptor (mAChR) stimulation with aceclidine prior to antigen injection. So, enhanced acetylcholine (ACh) signaling through mAChRs caused the severity of shock to increase. The same result could be obtained with the nAChR blocker hexamethonium. Thus, an increase in shock severity can be provided by enhancement of muscarinic stimulation with either muscarinic agonist or nicotinic antagonist [Nezhinskaya G.I., Nazarov P.G., Evdokimova N.R., Losev N.A., Saponov N.S., 2004]. Anaphylactic shock could be prevented with a combination of mAChR blocker methacine (applied 40 min prior) and AChE inhibitor neostigmine injected 15 min prior antigen. Purified plasma proteins influenced methacine activity differently. Purified human CRP co-administered with methacine blocked effect of the latter whereas normal human IgG injection prevented shock [Nezhinskaya G.I., Nazarov P.G., Evdokimova N.R., Losev N.A., Saponov N.S., 2004; Nezhinskaia G.I., Losev N.A., Nazarov P.G., Saponov N.S., 2005].

As shown in Figure 6, hexamethonium and methacine contain similar backbones and identical trimethylammonium heads in their molecules. It makes them very similar to both

phosphocholine and acetylcholine and leads to the suggestion that these drugs might be ligated by CRP as well. If so, CRP could affect their binding to appropriate AChRs and somehow modulate their signaling. The resulting data indicate that, whatever the mechanism of CRP interaction with these substances, the result of their joint action on basophils manifested in the form of enhancing the activity of the cells and more robust release of histamine.

Thus, the results of two studied FcγR-specific ligands, aggregated IgG and CRP, the reactant of acute phase of inflammation, have indicated common patterns of their influence on basophils, and features that distinguish them from each other. Common to aIgG and CRP is their activating effect on basophils. Both agents can activate normal basophils to the rapid release of histamine. Aggregated IgG is regarded as a model of immune complexes. Immune complexes are formed in many pathological and normal, physiological states. Our data suggest the ability of immune complexes to provide strong stimulus to basophils and possibly tissue mast cells.

Immune complexes containing IgG should evoke prompt release of vasoactive histamine and other proinflammatory mediators and cytokines produced by basophils. Consequently, in the sites of deposition of immune complexes (for example, in vessels impaired by atherogenesis, in kidney and joints affected with immune complex processes), basophils and mast cells should degranulate resulting in the release of their granules content and enhancement of its local impact on immunocompetent cells. It can be expected that the acute phase of inflammation, which is characterized by increasing the CRP gene expression and sharp strengthening of CRP protein production, will also be accompanied by significant increases in concentrations of histamine in the blood plasma and tissues due to the pentraxin activation of its release from the cells. It has been previously shown that CRP is synthesized not only by hepatocytes, but also by activated lymphocytes [Nazarov P.G., Sofronov B.N., 1983; Ikuta/Kuta, 1986]. Elevated local CRP production by activated lymphocytes and the related increased degranulation of basophils (mast cells) should take place in the sites of inflammation. According to the data presented herein, CRP and aggregated IgG show similar action on basophils in the absence of cholinergic drugs brought from outside. The difference between CRP and aggregated IgG is manifested only in artificial conditions, when blockers of ACh receptors are added to the cell system. If the impact of aggregated IgG on human basophils to take over standard, then the unusual effects of CRP on these cells can be explained by the possessing a ligand-binding activity by the pentraxin and its ability of binding the cholinergic antagonists due to their similarity to its ligands. The situations where CRP can meet such substances, are possible in the clinic, with medicines that contain in its structure quaternary ammonium base. In these cases, if there is an acute inflammation (and high CRP in the blood), perverse reactions may develop to such drugs because of their interaction with CRP and the CRP impact on their signaling (and therapeutic) functions.

5. Joint effects of CRP with aIgG and antibody to CD16

The data presented on Figure 7 shows the impact on the basophil secretory activity of three ligands, reacting with Fc-gamma receptors: aggregated IgG, C-reactive protein and monoclonal antibody to the Fc-gamma-type receptor III (low affinity). All three agents cause significant strengthening of histamine secretion (for all cases $p < 0.05$), of which IgG - most expressed.

With the incubation of the cells with two activators at once, CRP and aIgG, there was no addition of their effects. Instead, there was a decrease in histamine secretion compared not only with the expected mean of their separate effects (not shown), but also with the effect of any of these agents.

This may indicate that CRP and aIgG interact with the same receptors on the surface of basophils and compete with each other for cell surface binding sites or for intracellular messengers.

Some data indicates that CRP interacts with almost all types of FcγRs, including FcγRIIb. It has been reported that CRP binds leukocyte FcγRIIa (CD32) [Bharadwaj D. et al., 1999]. CRP induces ICAM-1, VCAM-1 and IL-8 up-regulation and down-regulates endothelial NO synthase (eNOS) via CD32 and CD64 and through NF-κB-mediated pathway [Yao-Jen Liang et al., 2006; Devaraj S. et al., 2005]. CRP up-regulates monocyte endothelial adhesion by activation of NF-κB through engaging the FcγRs CD32 and CD64, but not CD16. Biotinylated CRP bound to cells was colocalized with CD32 and CD64, but not with CD16 [Devaraj S., Davis B., Simon S.I. et al., 2006]. Also, preincubation with anti-CD32 and CD64 antibodies significantly inhibited binding of CRP to human aorta endothelial cells whereas antibodies to CD16 had no effect [Devaraj S., 2005]. FcγRIIs are thought to be the principal high affinity receptors for CRP expressed in endothelium. CRP prevents eNOS activation in cultured endothelium by a proper agents due to the interaction with FcγRIIb and inhibition of PP2A. In cells not expressing FcγRIIb, CRP did not antagonize eNOS activation while in cells expressing FcγRIIb, CRP blunted eNOS activation, indicating that the action of CRP requires FcγRIIb [Mineo C. et al., 2005].

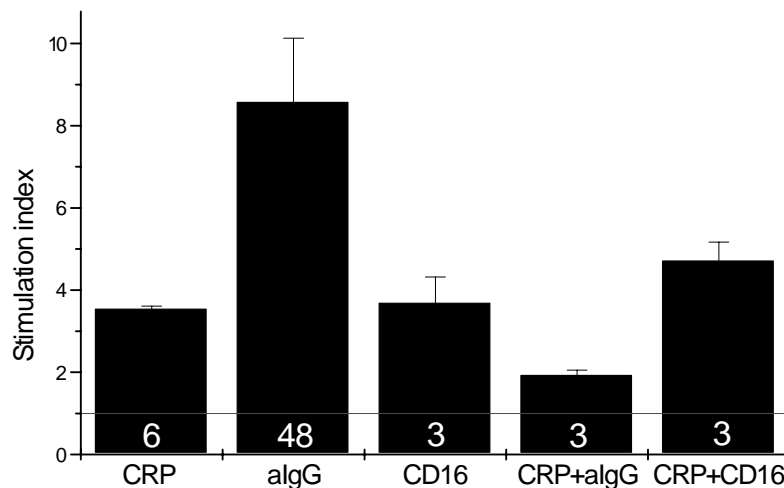


Figure 7. Human blood basophil responses to CRP, aIgG and anti-CD16, and their combinations.

CRP – human CRP, 50 $\mu\text{g/ml}$; aIgG – 100 $\mu\text{g/ml}$, mouse anti-human CD16 – 20 $\mu\text{l/ml}$. Numbers at the bottom of the columns represent the number of experiments. Means \pm SEM are plotted. The red control line represents the mean spontaneous histamine release in the presence of PBS taken to be 1.0.

Among Fc γ R₂s, the Fc γ R₂b contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its intracellular part, which can inhibit activation after coligation with ITAM containing Fc γ R₂s (all Fc γ R₂s except Fc γ R₂b). In humans, Fc γ R₂a is an activation receptor, while Fc γ R₂b is an inhibitory receptor [Daeron M., 1997; Ravetch J.V., Bolland S., 2001]. The existence of ITIM-containing Fc γ R₂b for IgG has been shown on mast cells and basophils as well [Katz H.R., 2002]. Cross-linking of Fc γ R₂b with Fc ϵ RI leads to inhibition of basophil triggering [Wiggington S.J. et al., 2008] and can inhibit the release of allergic mediators characteristic of type I hypersensitivity reactions.

Thus, Fc γ R₂b could be implicated in modulating human basophil secretory activity. Activation of Fc γ R₂b could occur under the joint action on basophils of aIgG and CRP as well as of aIgG and AChR antagonists (see Section 3, figure 4). In the latter case the activation of this suppressive receptor subtype may occur as a result of its coligation with nicotinic or muscarinic AChRs.

In contrast, basophil response to the joint action of CRP and anti-CD16 antibody did not show any inhibition. Instead, CRP and anti-CD16 effects were partially summarized. This result

favors the view that CRP does not interact with CD16, and thus low affinity FcγRIIIb are not the main binding sites of this pentraxin on the blood basophils. The cross-linking of FcγRs by CRP on the one hand and CD16 molecules by antibodies on the other seems to occur independently and activates independent signaling mechanisms leading to basophil activation. So, under simultaneous stimulation of the cells with these two activators (e.g. CRP and anti-CD16) additive stimulatory effect was observed.

6. Normal human blood basophil responses to repeated activation *in vitro*

Currently, there is little data on the survival of normal blood basophils in culture and ability to resynthesize their mediators *de novo* [Dvorak A.M. et al., 1982, 1985]. Information about normal basophil recovery/regranulation *in vitro* under activation through FcγRs by ligands like aggregated IgG and CRP we could not find in the literature. The study of this issue was the aim of our work. We have compared the survival of basophils during the cultivation of leukocytes *in vitro*, and investigated the ability of human blood basophils to degranulate repeatedly with re-release of histamine *in vitro*.

As our experiments showed, healthy donor blood basophils remain alive in leukocyte culture and able to produce histamine even 2-3 days after the beginning of cultivation *in vitro*.

The first stimulation of basophils was applied immediately after the start of cultures and lasted for 40 min. The cultures were stimulated with normal human heat aggregated IgG (50 or 500 μg/ml), human CRP (50 μg/ml), concanavalin A (10 or 50 μg/ml) or carbachol (1 μg/ml). Control cultures received PBS. After 40 min at 37°C supernatants were removed for histamine determination. This first basophil activation resulted in significant histamine release in response to aIgG, ConA, CRP and carbachol. The most active stimulant was carbachol (Figure 8). After that activated and control cells were washed thrice with PBS under centrifugation at 300 g for 10 min, and the last change of washing fluid was collected for histamine control. As was determined, it contained no measurable quantity of histamine. Then the cells were resuspended in fresh medium and cultivated for further 24 hr without stimulants. After 24 hours the activation of the cultures was repeated by adding the same stimulants. The cells were incubated with them for 40 min and supernatants were again collected to measure histamine release. Results of the first and second (at 24 hr) activation of basophils are shown in Figure 8.

As can be seen from Figure 8, after 24 hours of the start of cultivation the spontaneous basophil degranulation increased and histamine release was elevated. Significant histamine release at this term was induced by both doses of aIgG, CRP and ConA. The response to carbachol was not observed.

There are published data, which show that basophils in culture can live for a week. The authors of this observation have cultured cells in the presence of recombinant IL-3, a factor known to support basophil differentiation. According to their suggestion, IL-3 ensured basophils survival *in vitro* during that period [Yamaguchi M. et al., 1996]. However, the role of IL-3 in basophil survival is hard to judge based on the reported data, because the authors have not showed the data on basophil survival in cultures without adding IL-3. Unlike the work of Yamaguchi, we cultured cells without adding IL-3.

Carbachol is a nonmetabolizable analog of Ach, which possesses the same as ACh signaling activity, but differs from the Ach so that is not subjected to splitting enzymes. Carbachol is commonly used in experiments *in vitro* as a convenient substitute for Ach [Jensen A.A. et al., 2003].

We have obtained data that confirm the fact that basophils like mast cells [Falcone F.H. et al., 2006] are sensitive to cholinergic mediators and respond to carbachol with increased secretion of biologically active substances. Leukocytes responded *in vitro* to the first incubation with the carbachol with high elevation of histamine release. However, on the second day of cultivation, the cells showed no response to carbachol, although did respond significantly to the repeated stimulation with other activators (aggregated IgG, CRP and ConA).

Perhaps in this case the known phenomenon of desensitization was observed that is the loss of receptor sensitivity at re-exposition to the agonist [Picciotto M.R. et al., 2008]. Two types of cells desensitization has been described: homologous – when the cells no longer respond to the repeated impact of the same activator, which was used first time, and heterologous – when the cells do not respond to any reactivating stimulant. In our experiments, homologous desensitization to carbachol was observed: basophils that degranulated in response to the first contact with this carbachol, did not respond to reactivation with this agent.

At the same time, there was no distinct loss of basophil reactivity to repeated activation with plant lectin ConA, aggregated IgG and CRP. This indicates that the mechanisms of desensitization, described for neurotransmitters and their receptors, may not be used by other receptors and their signaling pathways (in our case mannose containing receptors of ConA and FcγRs). Alternatively, desensitization mechanisms could be compensated with some biochemical factors that do not affect the signaling of ACh receptors.

Thus, it can be concluded that basophils survive in culture for at least 24 hours and retain the ability to restore histamine and release it repeatedly in response to reactivation.

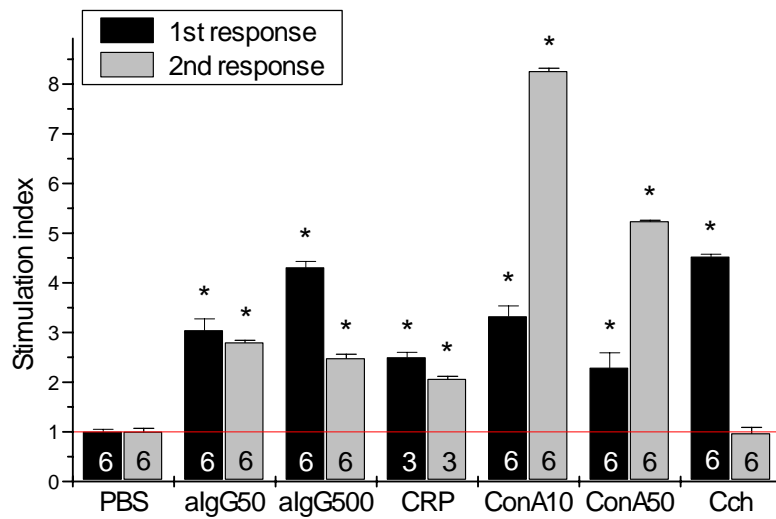


Figure 8. Normal human blood basophil responses to repeated activation by aggregated IgG, CRP and concanavalin A in vitro.

Means and standard errors of mean are shown. Normal human aggregated IgG was used in two doses – 50 and 500 $\mu\text{g/ml}$, CRP in a dose of 50 $\mu\text{g/ml}$, concanavalin A – in doses of 10 and 50 $\mu\text{g/ml}$, carbachol – 1 $\mu\text{g/ml}$. Numbers at the bottom of the columns represent the number of cultures. Asterisk – significantly different from the effect of PBS ($p < 0.05$ or less).

Basophils completed degranulation and histamine release upon stimulation with Fc γ R-specific ligands such as aggregated IgG and CRP or mannose-specific ligand ConA can restore their histamine content overnight to secrete it again at restimulation with the same factors. After cholinergic activation of basophils by carbachol the desensitization develops, which manifests itself in the loss of the cell ability to respond to the repeated exposure to this activator.

Acknowledgement

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References

Beekman J.M., Bakema J.E., van de Winkel J.G., Leusen J.H. Direct interaction between FcγRI (CD64) and periplakin controls receptor endocytosis and ligand binding capacity. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101(28):10392-10397.

Bharadwaj D., Mold C., Markham E., Du Clos T.W. Serum amyloid P component binds to Fcγ receptors and opsonizes particles for phagocytosis. *J. Immunol.* 2001; 166(11):6735–6741.

Bharadwaj D., Stein M.P., Volzer M., Mold C., Du Clos T.W. The major receptor for C-reactive protein on leukocytes is Fcγ receptor II. *J. Exp. Med.* 1999; 190(4):585-590.

Bühning H.J., Streble A., Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int. Arch. Allergy Immunol.* 2004; 133(4):317-329.

Devaraj S., Davis B., Simon S.I., Jialal I. CRP promotes monocyte-endothelial cell adhesion via Fc-γ receptors in human aortic endothelial cells under static and shear flow conditions. *Am. J. Physiol. Heart Circ. Physiol.* 2006; 291(3):H1170–H1176.

Devaraj S., Du Clos T. W., Jialal I. Binding and internalization of C-reactive protein by Fcγ receptors on human aortic endothelial cells mediates biological effects. *Arterioscler. Thromb. Vasc. Biol.* 2005; 26(7):1359-1363.

Du Clos T.W., Mold C. C-reactive protein an activator of innate immunity and a modulator of adaptive immunity. *Imm. Res.* 2004; 30(3): 261–277.

Du Clos T.W., Mold C. The role of C-reactive protein in the resolution of bacterial infection. *Curr. Opin. Inf. Dis.* 2001; 14(3):289-293.

Dvorak A.M., Colvin R.B., Monahan R.A. Immunoferritin electron microscopic studies with antibasophil serum of guinea pig basophil degranulation and regranulation in vitro. *Clin. Immunol. Immunopathol.* 1985; 37(1):63-76.

Dvorak A.M., Galli S.J., Morgan E., Galli A.S., Hammond M.E., Dvorak H.F. Anaphylactic degranulation of guinea pig basophilic leukocytes. II. Evidence for regranulation of mature basophils during recovery from degranulation in vitro. *Lab. Invest.* 1982; 46(5):461-475.

Falcone F.H., Zillikens D., Gibbs B.F. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. *Exp. Dermatol.* 2006; 15(11):855-864.

Fujimoto T., Sato Y., Sasaki N., Teshima R., Hanaoka K., Kitani S. The canine mast cell activation via CRP. *Biochem. Biophys. Res. Commun.* 2003; 301(1):212–217.

Grützkau A., Smorodchenko A., Lippert U., Kirchhof L., Artuc M., Henz B.M. LAMP-1 and LAMP-2, but not LAMP-3, are reliable markers for activation-induced secretion of human mast cells. *Cytometry A*. 2004; 61(1):62-68.

Gupta N., Scharenberg A.M., Fruman D.A., Cantley L.C., Kinet J.-P., Long E.O. The SH2 domain-containing inositol 5-phosphatase (SHIP) recruits the p85 subunit of phosphoinositide 3-kinase during FcγRIIb1-mediated Inhibition of B cell receptor signaling. *J. Biol. Chem.* 1999; 274(11):7489–7494.

Horowitz J., Volanakis J.E., Briles D.E. Blood clearance of *Streptococcus pneumoniae* by C-reactive protein. *J. Immunol.* 1987; 138(8):2598–2603.

Ikuta T., Okubo H., Ishibashi H., Okumura Y., Hayashida K. Human lymphocytes synthesize C-reactive protein. *Inflammation*. 1986; 10(3):223–232.

Jensen A.A., Mikkelsen I., Frølund B., Bräuner-Osborne H., Falch E., Krosgaard-Larsen P. Carbamoylcholine homologs: novel and potent agonists at neuronal nicotinic acetylcholine receptors. *Mol. Pharmacol.* 2003; 64(4):865-875.

Katz H.R. Inhibitory receptors and allergy. *Curr. Opin. Immun.* 2002; 14(6):698-704.

Kawashima K., Fujii T. Extraneuronal cholinergic system in lymphocytes. *Pharmacol. Ther.* 2000; 86(1):29-48.

Kirkpatrick C.J., Bittinger F., Nozadze K., Wessler I. Expression and function of the non-neuronal cholinergic system in endothelial cells. *Life Sci.* 2003; 72(18-19):2111-2116.

Knol E.F., Mul F.P., Jansen H., Calafat J., Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J. Allergy Clin. Immunol.* 1991; 88(3 Pt 1):328-338.

Lange A., Karabon L., Tomeczko J. IL-6- and IL-4-related proteins (C-reactive protein and IgE) are prognostic factors of asbestos-related cancer. *Ann. N.Y. Acad. Sci.* 1995; 762:435-438.

Liang Y.-J., Shyu K.-G., Wang B.-W., Lai L.-P. C-reactive protein activates the nuclear factor-κB pathway and induces vascular cell adhesion molecule-1 expression through CD32 in human umbilical vein endothelial cells and aortic endothelial cells. *J. Mol. Cell. Cardiol.* 2006; 40(3):412-420.

Marnell L.L., Mold C., Volzer M.A., Burlingame R.W., Du Clos T.W. C-reactive protein binds to Fc gamma RI in transfected COS cells. *J. Immunol.* 1995; 155(4):2185-2193.

Metzelaar M.J., Wijngaard P.L., Peters P.J., Sixma J.J., Nieuwenhuis H.K., Clevers H.C. CD63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J. Biol. Chem.* 1991; 266(5):3239-3235.

- Mineo C., Gormley A.K., Yuhanna I.S., Osborne-Lawrence S., Gibson L.L., Hahner L., Shohet R.V., Black S., Salmon J.E., Samols D., Karp D.R., Thomas G.D., Shaul P.W. FcγRIIB mediates C-reactive protein inhibition of endothelial NO synthase. *Circ. Res.* 2005; 97(11):1124-1131.
- Mohamed H.A., Mosier D.R., Zou L.L., Siklós L., Alexianu M.E., Engelhardt J.I., Beers D.R., Le W.D., Appel S.H. Immunoglobulin Fc gamma receptor promotes immunoglobulin uptake, immunoglobulin-mediated calcium increase, and neurotransmitter release in motor neurons. *J. Neurosci. Res.* 2002; 69(1):110-116.
- Mold C., Gresham H.D., Du Clos T.W. Serum amyloid P component and C-reactive protein mediate phagocytosis through murine FcγRs. *J. Immunol.* 2001; 166(2):1200–1205.
- Nakayama S., Du Clos T.W., Gewurz H., Mold C. Inhibition of antibody responses to phosphocholine by C-reactive protein. *J. Immunol.* 1984; 132(3):1336–1340.
- Nazarov P.G. *Reactants of Acute Phase of Inflammation.* 2001; Nauka, St. Petersburg. 423 pp. (In Russian).
- Nazarov P.G., Krylova I.B., Evdokimova N.R., Nezhinskaya G.I., Butyugov A.A. C reactive protein: a factor of inflammation binding and inactivating acetylcholine. *Cytokines and Inflammation.* 2006; 6(4):32-35. (in Russian).
- Nazarov P.G., Krylova I.B., Evdokimova N.R., Nezhinskaya G.I., Butyugov A.A. C-reactive protein: a pentraxin with anti-acetylcholine activity. *Life Sci.* 2007; 80(24-25):2337–2341.
- Nazarov P.G., Nezhinskaya G.I., Krylova I.B. Pentraxins: antiarrhythmogenic factors of inflammation. *Ann. Arrhythmol.* 2005; 2(suppl):20.
- Nazarov, P.G., Sofronov, B.N. Synthesis of C-reactive protein by lymphoid cells. In: *Mediators of Immune Response in Experiments and Clinics.* Institute of Immunology, USSR Ministry of Health, Moscow, 1983; 112–113. (In Russian).
- Nezhinskaya G.I., Losev N.A., Nazarov P.G., Saprionov N.S. Effect of acetylcholine and C-reactive protein on regulation of anaphylactic shock in guinea pigs. *Eksp. Klin. Farmakol.* 2005; 68(4):49-52. (In Russian)
- Nezhinskaya G.I., Nazarov P.G., Evdokimova N.R., Losev N.A., Saprionov N.S. Cholinergic regulation of anaphylactic shock: impact of C-reactive protein. *Cytokines and Inflammation.* 2004; 3(1): 39-41. (in Russian).
- Nieuwenhuis H.K., van Oosterhout J.J., Rozemuller E., van Iwaarden .F, Sixma J.J. Studies with a monoclonal antibody against activated platelets: evidence that a secreted 53,000-molecular weight lysosome-like granule protein is exposed on the surface of activated platelets in the circulation. *Blood.* 1987; 70(3):838-845.

Oishi K., Yoshizumi T., Seki N., Uchida M.K. Carbachol-induced membrane ruffling and its desensitization in rat basophilic leukemia (RBL-2H3) cells transfected with m3 muscarinic acetylcholine receptors. *Biol. Pharm. Bull.* 2004; 27(10):1544-1548.

Picciotto M.R., Addy N.A., Mineur Y.S., Brunzell D.H. It is not "either/or": Activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog. Neurobiol.* 2008; 84(4):329-342.

Ravetch J.V., Bolland S. IgG Fc receptors. *Annu Rev Immunol.* 2001 ; 19 :275–290.

Suresh M.V., Singh S.K., Agrawal A. Interaction of calcium-bound C-reactive protein with fibronectin is controlled by pH: in vivo implications. *J. Biol. Chem.* 2004; 279(50):52552-52557.

Suzuki K., Kitani S., Takaishi T., Ito K., Ra C., Morita Y. Nonreleasing basophils convert to releasing basophils by culturing with IL-3. *J. Allergy Clin. Immunol.* 1996; 97(6):1279-1287.

Szalai A.J., Briles D.E., Volanakis J.E. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in transgenic mice. *J. Immunol.* 1995.; 155(8):2557-2563.

Tseng J., Mortensen R.F. Binding of human C-reactive protein (CRP) to plasma fibronectin occurs via the phosphorylcholine-binding site. *Mol. Immunol.* 1988; 25(8):679-686.

Tseng J., Mortensen R.F. The effect of human C-reactive protein on the cell-attachment activity of fibronectin and laminin. *Exp. Cell Res.* 1989; 180(2):303-313.

Van de Winkel J.G., Capel P.J. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol. Today.* 1993; 14(5):215–221

Van de Winkel J.G.J., Anderson C.L. Biology of human immunoglobulin G Fc receptors. *J. Leukoc. Biol.* 1991; 49(5):511–524.

Wessler I., Kilbinger H., Bittinger F., Unger R., Kirkpatrick C.J. The non-neuronal cholinergic system in humans: expression, function and pathophysiology. *Life Sci.* 2003; 72(18-19):2055-2061.

Wigginton S.J., Furtado P.B., Armour K.L., Clark M.R., Robins A., Emara M., Ghaemmaghami A.M., Sewell H.F., Shakib F. An immunoglobulin E-reactive chimeric human immunoglobulin G1 anti-idiotypic inhibits basophil degranulation through cross-linking of FcεRI with FcγRIIb. *Clin. Exp. Allergy.* 2008; 38(2):313-319.

Xiao Y., Kellar K. J. The comparative pharmacology and up-regulation of rat neuronal nicotinic receptor subtype binding sites stably expressed in transfected mammalian cells. *J. Pharmacol. Exp. Ther.* 2004; 310(1):98–107.

Zhang D., Sun M., Samols D., Kushner I. STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. *J. Biol. Chem.* 1996; 271(16):9503–9509.