Effect of Some Cholinergic Substances on Spontaneous Immunoglobulin E Production by Mononuclear Cells of the Peripheral Blood in Hay Fever Patients

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The effect of carbachol, forskolin, and cycloheximide on IgE biosynthesis is studied in hay fever patients. The effect of carbachol is noted only for significant synthesis of IgE *de novo*. The administration of forskolin causes the inhibition of both IgE and IgG spontaneous production, while subsequent administration of carbachol neutralizes this effect. When *de novo* IgE synthesis is absent, the degradation of IgE in the culture of peripheral blood mononuclear cells decreases significantly in the presence of cycloheximide.

Key Words: *immunoglobulin E; spontaneous production; cholinoreceptors*

Cholinoreceptive structures have been found on peripheral blood lymphocytes in heathy people [5,9] and in hay fever sufferers [1]. Proximity and interaction of M-cholinoreceptors with the structures binding antigens (allergens) are noted in hay fever patients [1]. Although IgE class antibodies are known to play a pathogenic role in the development of human allergic disorders, it is unclear whether the interaction of transmitter and immune receptors affects the biosynthesis of IgE and what implication this has for the development of allergic disorders.

The aim of the present investigation was to study the effect of some cholinomimetics on the biosynthesis of IgE in hay fever patients.

MATERIALS AND METHODS

The patients were studied under conditions of sensitization to pollen of trees and grasses at the "peak" (maximum dose of allergen) of specific immunotherapy with causally significant allergens. The method of determination of spontaneous production of IgE in a culture of peripheral blood mononuclears (PBM) was used *in vitro* due to its informativeness, reported by a number of scientists, for assessment of the IgE response *in vivo* in hay fever patients [7]. The culturing of PBM was performed as described elsewhere [2]. The total amount of IgE in supernatant of the PBM culture and of IgE bound with cells at the end of culturing was denoted as the total production of IgE *in vitro*. *De novo* IgE synthesis in culture was determined by subtracting from this value the preformed IgE amount (contained in cells prior to the start of culturing). Spontaneous production of IgE was studied similarly for comparison.

The cholinomimetic carbachol was added to the PBM culture at the start of culturing in a concentration of 10^{-3}-10^{-11} M. The level of IgE was determined on the 3rd and 7th days of PBM culturing using the immune enzyme technique [3].

RESULTS

An effect of carbachol on spontaneous production of IgE in a PBM culture could be observed only

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TABLE 1. Effect of Carbachol on the Level of Spontaneous Production of IgE and IgG in PBM of Patients ($M \pm m$)

Index	Specific immunotherapy with tree and grass pollen				Pollination season		Specific immunotherapy with insect allergens	
	Time of PBM culturing, days							
	3	7	3	7	3	7	7	7
	IgE, pg/2×10⁶ PBM							
Control	1980±493	1916±192	6952±418	8891±182	3241±1130	1531±195	1710±277	
De novo IgE synthesis	308	244	5509	7448	601	416	595	
With carbachol	1888±184	2072±440	5592±179	7936±477	3624±470	1410±123		
With forskolin							1270±260	
With carbachol + forskolin							1621±26	
	IgG, ng/2×10⁶ PBM							
Control	1639±54	1756±285	866±114	1148±47	2146±419	2336±416	2046±662	1236±96
De novo IgG synthesis	726	843	552	834	1337	1737	1626	816
With carbachol	2007±165	2149±64	924±13	1164±160	2114±305	2330±15	1520±255	
With forskolin								631±226
With carbachol + forskolin								523±46

for substantial *de novo* synthesis. *De novo* IgE synthesis in excess of 200 pg was considered substantial [6]. Preliminary investigations did not reveal a dose-dependent effect of carbachol on spontaneous production of IgE. This was 7317-7736 pg/2×10⁶ PBM in the concentration range of carbachol 10^{-3}-10^{-11} M, whereas in the control it was 9917 pg/2×10⁶ PBM for substantial *de novo* IgE synthesis, being equal to 5740 pg/2×10⁶ PBM (the level of preformed IgE was 4177pg/2×10⁶ PBM, $p=0.03$ in comparison with the control).

The effect of carbachol on spontaneous IgE and IgG production in PBM is presented in Table 1. Substantial *de novo* IgE synthesis was found as early as on the 3rd day of PBM culturing. The diverse effect of carbachol on the level of spontaneous IgE production comprised both inhibition and stimulation of it.

The effect of carbachol on spontaneous IgG production in PBM was the direct opposite of that on IgE production, as well as the value of IgG production itself.

Only an inhibitory effect of carbachol on spontaneous IgE and IgG production in PBM was observed in insect allergy, studied for comparison. Forskolin (Calbiochem, Behring Corp., La Jolla) addition at 10^{-5} M to PBM culture induced inhibition of spontaneous production of both IgE and IgG of PBM. Carbachol added with forskolin to PBM culture neutralized its action, bringing the IgE level close to the control value. Forskolin has an antiproliferative effect [4] due to the inhibition of activated B cell transition from the G_1 phase to the S phase. Therefore, both IgE and IgG spontaneous production is suppressed. The abolishment of the effect of forskolin on carbachol-stimulated IgE synthesis may be explained by forskolin boosting of cAMP synthesis whereas, carbachol raises the cGMP level in the cytoplasm of cells.

Degradation of IgE was significantly decreased in the presence of cycloheximide in PBM culture when *de novo* IgE synthesis was absent in a patient with sensitization to tree pollen examined during the susceptible period [8]. The control *de novo* IgE synthesis comprised 406 and 277 pg/2×10⁶ PBM in a culture with cycloheximide at 50 μg/ml. The degradation of IgE in the presence of carbachol was similar to the control and *de novo* IgE synthesis comprised 491 pg/2×10⁶ PBM. However, while *de novo* IgE synthesis was absent, a significant increase of the total IgG production and *de novo* IgG synthesis, which was reliably inhibited by the administration of cycloheximide in PBM culture, was noted in this patient. Cycloheximide is known to inhibit protein synthesis, and is able, notably, to block immunoglobulin production in a lymphocyte culture. No effect of carbachol on IgG production has been noted.

Thus, the findings do not provide a clear picture of how cholinergic and transmitter receptors interact in the model of spontaneous IgE and IgG production.

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Effect of New Muramyl Dipeptide Derivatives on the Major Components of Immunity

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Immunomodulatory activity of five new synthetic muramyl dipeptide (MDP) derivatives (β-heptylglycoside-MDP, β-hexadecylglycoside-MDP, polyacrylamide-MDP, polyacrylamide-MDP-phosphatidylethanolamine, and dexal-MDP) is studied *in vitro* in different test systems.

Key Words: *muramyl dipeptide derivatives; immunomodulation*

MDP derivatives and glucosamine-MDP have been intensively studied during the last decade, but only a few preparations have been used in clinical practice, notably, in oncology [10]. Monotherapy with MDP derivatives is not effective enough. However, these preparations are of interest as components of complex therapy. The rather high toxicity of MDP, which is due to stimulated production of tumor necrosis factor (TNF), interleukin-1 (IL-1), and prostaglandins, is an obstacle impeding their clinical use. New analogs of natural MDP, which will be less toxic and more effective in the activation of regulator and effector lymphocytes, are required.

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The aim of our study was to investigate five new MDP derivatives synthesized by us [2,6]: β-heptylglycoside-MDP (C_7H_{15}MDP); β-hexadecylglycoside-MDP ($C_{16}H_{33}$MDP); polyacrylamide-MDP (PMDP); polyacrylamide-MDP-phosphatidylethanolamine (PMDP-PE); and dexal-MDP (DMDP) in different test systems *in vitro*.

MATERIALS AND METHODS

C57Bl/6 and DBA/2 mice of both sexes were used in the study. Medium RPMI-1640 containing 5% fetal calf serum, 50 μg/ml gentamicin, 2 mM L-glutamine, 10 mM HEPES, and 5×10^{-5} M 2-mercaptoethanol was used in the experiments *in vitro*. MDP derivatives were used in the tests in equimolar concentrations: 5, 10, and 20 μM. C57Bl/6

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