

# Antiallergic drugs and the immune response

Interactions and possible clinical relevance

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Since the pharmacological treatment of allergic diseases is used in patients with alteration of the immune response due to atopy and possible concomitant infections, we have investigated the possible effects of such drugs as cromolyn, theophylline, ketotifen, oxatomide, astemizole, fenoterol, pirenzepine, and rosaprostol on the *in vitro* immune response, in order to obtain experimental data and, as a consequence, clinical conclusions. In a series of investigations by our group and other authors the following immunological parameters have been considered: T cell activation induced by different pathways (i.e. autologous stimulation, PHA, anti-CD3, -CD2 and -CD28 monoclonal antibodies), and lymphokine production (i.e. IL-1, IL-2 and gamma-IFN). For a more detailed experimental model the experiments have been performed both in bulk culture and by using T cell clones derived from the peripheral blood. The results show cromolyn to have an enhancing effect, theophylline and ketotifen a suppressing effect, whereas the remainder show no effect on the immune response. These data are considered and discussed from the aspect of their possible clinical relevance and also in light of the *in vivo* data previously reported.

**Key words:** allergic diseases; antiallergic drugs; immune response.

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The pharmacological treatment of respiratory and alimentary allergic diseases comprises drugs, such as cromolyn, theophylline, B<sub>2</sub> mimetics, glucocorticosteroids, antihistamines (both anti-H<sub>1</sub> and anti-H<sub>2</sub> receptors), but also cytoprotective compounds, as recently described (5).

These drugs are employed in patients with alteration of the immune response due to atopy (2, 3) and possible concomitant infections. However, since previous studies have shown interaction between glucocorticosteroids, theophylline, ketotifen, or adrenergic agents and the immune system (1, 9, 11, 12, 24, 26, 27, 32), we considered it useful to investigate the possible effects of other compounds employed in these disorders in order to obtain additional experimental data concerning these same drugs and, consequently, their proper clinical use.

We recently demonstrated that cromolyn (4),

ketotifen (6) and theophylline (10, 32), commonly used in the treatment of allergic syndromes, can interfere with the *in vitro* immune response, whereas oxatomide (7), fenoterol (8) and astemizole (manuscript in preparation) do not. In addition, we have studied two drugs with cytoprotective activity i.e. pirenzepine (anti-M1 receptor antagonist) and rosaprostol (prostaglandin analogue), because they have been demonstrated useful in the treatment of food allergy patients (5). Interestingly, pirenzepine does not influence the number and distribution of gastroduodenal mast cells, a cellular population highly represented in the digestive tract in normal conditions (16). On the other hand, since it is well known that in this syndrome prostaglandins suppress the immune response (13), we also studied a prostaglandin analogue (rosaprostol).

In the present review the effects of these

Table 1  
T cell proliferation and lymphokine production

| Drugs        | T cell proliferation |          |          |         |          | Lymphokine production |       |
|--------------|----------------------|----------|----------|---------|----------|-----------------------|-------|
|              | PHA                  | CD3      | CD2      | CD28    | AMLR     | IL-2                  | IFN-γ |
| Cromolyn     | + 56 **              | + 39 *** | + 38 *** | + 54 ** | + 200 ** | -28 *                 | -15   |
| Ketotifen    | + 18                 | n.d.     | n.d.     | n.d.    | -29 ***  | n.d.                  | n.d.  |
| Oxatomide    | -3                   | -2       | -1       | n.d.    | n.d.     | + 13                  | -2    |
| Astemizole   | + 5                  | -9       | -7       | -16     | n.d.     | -5                    | + 5   |
| Theophylline | -42 ***              | -48 ***  | + 7      | n.d.    | n.d.     | -10                   | -13   |
| Fenoterol    | + 4                  | -2       | -1       | -12     | n.d.     | n.d.                  | n.d.  |
| Pirenzepine  | + 25                 | n.d.     | n.d.     | n.d.    | -15      | -9                    | -7    |
| Rosaprostol  | + 15                 | -5       | + 32     | + 5     | n.d.     | -3                    | + 6   |

T cell proliferation data are reported as percentage (+ 0-) of proliferation vs. proliferation without the drug.  
Student's t test: \*\*\*  $P < 0.001$ , \*\*  $P < 0.05$ , \*  $P < 0.01$ .  
Lymphokine production: data reported as described above.

drugs on the immune response will be reported in detail and the possible clinical perspectives considered.

The experimental approach consisted in investigating the possible effects of the above-mentioned compounds on T cell proliferation, lymphokine production and T cell clones. We investigated T cell proliferation because T cells play a crucial role in the regulation of the immune response network (i.e. T cell subsets with regulatory helper or suppressor functions and lymphokine production) and also because our group has long been primarily involved in this specific line of research.

We studied the different activation pathways in T cell proliferation, using both anti-CD3 monoclonal antibody (MAb) and phytohemagglutinin (PHA). The former acts through the complex CD3/Ti (T cell receptor, i.e. the specific receptor which recognizes the antigen) and the latter is shown to be effective mainly through a membrane responsive structure whose nature is still unknown, although in special culture conditions (i.e. under limiting dilution or micromanipulation) this mitogen is capable of inducing proliferation of virtually 100 % of T cells (20).

We also studied another pathway of T lymphocyte activation, i.e. through the CD2 molecule complex (17). This membrane structure is also well known as the sheep erythrocyte receptor, which was the first T cell surface marker. This activation pathway differs from the CD3/Ti com-

plex as far as the second messenger is concerned, as recently described (21).

Finally, we employed another MAb (CD28) acting through a different pathway (21).

We further studied the effect of these drugs in the autologous mixed lymphocyte reaction (AMLR), whose functional responsive structure is related to the 1α-AGP (alpha-1-acid glycoprotein or orosomucoid) (10), a protein which is highly increased in the first phase of inflammation, and thus, in the present context, thought to be involved in the phenomenon of immunophlogosis, relevant in allergy and adverse reactions to foods.

The lymphokine production induced by PHA (interleukin-2 (IL-2) and interferon (IFN)) and γ LPS (interleukin-1 (IL-1)) was also evaluated.

As in bulk cultures we had observed that T cell response was modified by certain compounds, we also studied the effect of these drugs on DNA synthesis of T cell clones, derived from the peripheral blood of normal subjects, stimulated with PHA or anti-CD3 MAb.

Experimental evidence

Our experimental procedures will not be described in this review as they have been previously reported (3, 4, 6, 7, 32).

The effect of *cromolyn* on the *in vitro* immune response was a significant increase in T cell proliferation. This was observed when *cromolyn* was added to T cells, stimulated by

PHA, anti-CD3, -CD2 and -CD28 MAbs and by AMLR. Cromolyn did not influence IL-1, IL-2 and IFN $\gamma$  production, but enhanced IL-2 receptor expression. At the clonal level cromolyn had no effect on IL-2 production, possibly due to overconsumption by T cells.

Theophylline significantly decreases both PHA- and anti-CD3- induced PMNC proliferation, whereas IL-2, IFN $\gamma$ - production and IL-1 production is not influenced by this compound. On the other hand, theophylline also shows an inhibitory effect on both the proliferation of T cell clones and their IL-2 production.

The data obtained with theophylline clearly show the importance of studying the *in vitro* effects in detail. A study of T cell clones compared with bulk cultures gives another experimental perspective. In bulk culture the coexistence of several different immunocompetent and functional cellular populations might influence the result concerning a single cytokine. In fact, a cytokine regularly produced by the competent cellular population could be utilized by the natural cellular target of this same cytokine. In other words, if we detect in supernatants of a bulk culture a reduced level of a certain soluble factor, we cannot affirm whether the reduction is due to decreased production or to enhanced uptake. Including a study of T cell clones in the experimental design provides valuable additional information.

Ketotifen does not affect PHA-induced PMNC proliferation, but decreases AMLR.

Oxatomide, astemizole, pirenzepine, rosaprostol and fenoterol can be defined as "inert" molecules regarding the *in vitro* effect on the immune response.

### Conclusion

For many years now there have been few investigations of the possible effects of anti-allergic drugs on the immune system. In general, when a drug is used over a longer period and not specifically for an immunological disorder, its eventual side effects, or even "additional" effects, on the immune response have remained uninvestigated. We therefore consider this line of immunopharmacological research of great importance for possible clinical relevance.

We consider that a detailed study should be made of the possible influence of pharmacologic anti-allergic compounds on the *in vitro* immune response. An understanding of the functional mechanisms involved might contribute to a more appropriate clinical use of these drugs.

A clear demonstration of this theory (both *in vitro* and *in vivo* is supported by studies concerning the calcium-blocker drugs e.g. nifedipine and verapamil) (22, 23, Morgano et al. submitted), theophylline (1, 32), ranitidine (9, 28) glucocorticosteroids (14, 33) and adrenergic agents (15, 24, 25, 36) on the immune system. Since these pharmacological compounds suppress the immune response (even *in vivo*), these drugs should be used with caution in patients with infections or with disorders of the immune response.

We are well aware that the *in vivo* studies are still largely unsatisfactory, nevertheless we believe that the *in vitro* evidence reported by us and others might provide the rationale for further *in vivo* and clinical investigations.

In this review we have reported our data regarding the effects on *in vitro* immunological parameters by: cromolyn, theophylline, ketotifen, oxatomide, astemizole, fenoterol, rosaprostol and pirenzepine. We observed an *in vitro* enhancing effect of cromolyn, an inhibitory effect of theophylline and ketotifen and an absence of effects with oxatomide, astemizole, fenoterol, rosaprostol and pirenzepine.

We would emphasize that the scientific rationales of our experimental studies have a sound basis since: 1) IFN $\gamma$  stimulates production of the secretory component of IgA (18, 29), 2) IL-2 and IFN $\gamma$  promote B lymphocyte growth and differentiation (19), 3) intestinal lymphocytes are hyporesponsive to mitogenic stimuli (19), and 4) IFN $\gamma$  synthesis is suppressed in allergic patients (31).

A more clinical consideration is that theophylline and ketotifen should be used with care in patients with infections. In addition, since the efficiency of the immune system decreases with age, it is clinically relevant to carefully evaluate the use of these compounds in older patients.

Our studies have further shown the theophylline inhibition of the immune response to be dose dependent, and more sophisticated experiments indicate that the "once a day" administration of theophylline (35), should be warmly advocated (12, 32).

Although it is premature to derive any clinical conclusions from this experimental evidence, we can speculate that cromolyn might play a synergistic role, cooperating with mucosal associated lymphoid tissue during specific immunotherapy in respiratory allergy or disorders related to food allergy (4). Our experimental data and speculations prompted us to consider the clinical importance of cromolyn. The drug, which is already known as an effective anti-allergic drug without side effects, is further revealed to potentiate the immune response. In our opinion this should be a further reason for considering this compound as first choice drug in the treatment of allergic disorders.

Further studies are already in progress in our laboratory: to verify these hypotheses and to confirm the detailed mechanisms of action of various drugs, particularly in allergy.

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