



Editorial

Again an asthma model: but a useful one

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Dr. Ramos-Barbón's¹ team's article, published in this issue of *ARCHIVOS DE BRONCONEUMOLOGÍA* did not pass by undetected by those of us who believe wholeheartedly in the added value of *in vivo* research with induced allergic asthma models in mice. Despite what detractors may say,² it seems obvious that in the last 10-15 years mice that have been sensitised to various antigens have helped us to understand the disease.³ The fact is that being a detractor or not often depends on how we direct our expectations. Those of us who believe that inducing "respiratory sensitivity" in mice reproduces patients' asthmatic process to a certain extent, and understand that all models obviously have certain limitations, tend to express interest.⁴ Some even profess enthusiasm, as we do.⁵ And then there are very purist researchers who would only accept an "asthmatic mouse", and it is true that such an achievement, which would necessarily go beyond creating a mere experimental model, has not been reached. It is therefore fortunate that numerous articles in prestigious journals have been published which propose experimental murine models for the study of allergic asthma.

Returning to the subject of the article, its authors practice what can be considered an extreme sport: daring to present yet another model of induced asthma in mice before the scientific community. Furthermore, it is induced by exposure to ovalbumin, an antigen which is now universally used. Their "daring" is not without a dash of intrigue: what do these authors provide? What is new? In our opinion, the novel element is somewhat hidden. It is found by reading between the lines, and is not clearly visible. One respectful criticism, therefore, is that the authors may not have framed the magnitude of their efforts clearly enough, and thus have not openly highlighted the added value of the model that they propose. In this brief editorial comment, we would like to focus on and analyse four aspects of the published data: a) the model's inducement process; b) the method and the purpose of evaluating the response; c) the results obtained, and d) interpretation and discussion of those results. Let us proceed part by part.

Sensitising and provoking attacks: reproducing severe asthma

With regard to the inducement process, the article proposes a severe asthma model in the mouse using prior intraperitoneal

sensitising followed by intranasal provocation with ovalbumin during 12 weeks, three times a week. The authors have overcome a long-standing barrier by using repeated exposure to ovalbumin: the development of immunological tolerance. This fact alone has merit and may be due, according to the authors, to the route of systemic exposure in the sensitising stage. However, we must recall that a team from the *Jiménez Díaz* Foundation induced remodelling by exposing animals to the antigen (also intensively: during 12 weeks, 3 days a week) exclusively through the respiratory tract, during both the sensitising and provoking phases.⁶ One of the keys to their success may lie in the use of A/J mice, since it has been seen that the development of remodelling depends greatly on the strain of mouse (it is more difficult to attain in BALB/c). Other groups have previously succeeded in inducing a chronic, irreversible process similar to that observed in patients, as the authors themselves point out,^{7,8} and even use similar inducement processes (the exposure route, antigen, mouse strain, sex, etc.). We do believe, however, that it would have been appropriate for the article's authors to contrast their data with those from a relatively recent study by Prof. Galli's team,⁹ as we will explain further in a later section. The efforts made by the researchers in the study in question have both merit and scientific logic, and cannot be reduced to a merely arithmetical matter: the intense exposure through the respiratory route brings the inducement process significantly closer to the natural/spontaneous process found in patients' severe or chronic asthma. It is likely that this intense antigenic stimulation explains some specific results (see below). We must call attention to this aspect of the protocol because other researchers have been working on more apparent, and of course, very important matters having to do with inducing respiratory sensitivity for a better approximation of the spontaneous disease in patients^{10,11} but "simply" intensifying exposure to the antigen is an undeniable breakthrough.

Evaluating the response of the airways

Once a model has been induced and we are looking for data with a biomedical impact, the decision regarding what is to be evaluated (variable waves to be measured) is an interesting one. In the article that concerns us, we are looking for a distinguishing feature in its evaluations of the response to ovalbumin. The authors emphasise the "development and integrated evaluation of inflammation and

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remodelling" as a special point of interest. It is certainly interesting to make parallel measurements of the inflammatory and remodelling processes, given that there are numerous unknowns in the underlying mechanisms. To our knowledge, other similar studies have already been cited,^{6,8,9} specifically those with chronic models induced with ovalbumin. In the article that concerns us, however, the measurements are more precise because they were taken using quantitative morphology, while in earlier cases, semi-quantitative scales were used (normally, the classic point or scoring system). This integrated quantitative evaluation is quite flexible, and certainly provides added value to the study. For example, it allows us to establish statistical correlations between the different variables for each of the processes (inflammation and remodelling). Although these correlations would not establish definitive biological relationships between variables, they can doubtless provide clues or point toward possible functional links, that is, illness mechanisms. This should be confirmed using later experimental studies. Therefore, some analysis is in this sense missing, but there is surely much to come. For example, is there a statistical correlation between the degree of eosinophilia and an increase in collagen? Or, perhaps even more interesting, is there one between the number of mast cells and muscle mass?

With regard to the phenomena being investigated, it is also interesting to see the distinction made between airways according to size (small, medium and large) and their anatomical location. This is uncommon in this type of study, but its impact is clear, and in a certain way indirectly affects the debate on the united airway theory. This is commented on briefly in the following section.

About the results

It is still important for us to evaluate how the model is induced and what it evaluates, but this holds no interest if no relevant data can be derived from that model. We see many results that must necessarily be presented in this type of article (regarding goblet cells, muscle mass, etc.), but the intention is to draw the reader's attention to two observations only: a) the number of mast cells and eosinophils, and b) the effects on airways of different sizes.

Evaluating the number of mast cells is not common except for research teams that specialise in that cell population.^{9,12} However, the authors measure it and show that these cells have a high degree of activity in the model which they propose. This is particularly relevant data, given the resurgence of interest in this cell type for allergic diseases. Their role is being assigned increasing relevance. Counting the mast cells that infiltrate the tissue, as these authors do, is a very accurate way of measuring the cells' activity. We however believe that the circle will only be closed with a de facto measurement of the production of specific mast cell mediators, such as protease-1 in mouse mast cells (mMCP-1) and others.^{13,14} Within the new proposed model, 10 times more mast cells are detected in sensitised mice than in non-sensitised ones (from 0.28 to 2.8/cm²), meaning that a change of that magnitude does occur. This requires special attention, given that in other cases, such as that described by Yu et al,⁹ there is an increase by up to four times. The model and the counting method are certainly different, but the difference is surprising, as it is generally even smaller. We cannot rule out the possibility of the intensive exposure protocol explaining the significant increase in the number of these cells. Although we do not observe a redistribution of mast cells toward smooth muscle tissue as we would have liked, given recent patient studies,¹⁴ it would be convenient to evaluate this cell population histologically and functionally in the model at a later date. It is surprising that the eosinophils would make up 50% of the total infiltrated cells in the lungs. They normally make up between 10 and 30%. This degree of eosinophil infiltration might be a good thing from a pharmacological or methodological point of view, since it will permit us to study

molecules' modulating effect on eosinophilic inflammation. One point which has been highly criticised in murine models is that the eosinophils that infiltrate the airways are not activated. Are they in this case? There is no doubt that since mast cells and eosinophils are two different populations pertaining to this type of process, the fact that particularly intense recruitment was achieved makes this model quite interesting if examined from the viewpoint of studying the activity of these specific cells.

The second aspect of the models' data to generate interest is how the small, medium-sized and large airways are affected. All show remodelling (Mtc and Mmex indices), which on the one hand literally reflects the scope of the antigen which reaches the most distal areas (with a smaller calibre). On the other hand, as stated before, it indirectly supports the united airway theory, which states that the problem is not limited to the upper or lower airways, but rather affects the entire tracheobronchial tree.¹⁵ The nasal mucosa was not studied here, but considering the results in the rest of the airways, it is likely to be affected as well.

Limitations of the study

Some of the article's weak points or limitations have already been pointed out above, and we will now raise other points. As the model is new, it lacks data on the respiratory function, and particularly on the bronchial hyperreactivity that the animals may suffer. While it is true that one must have access to equipment that can measure this function, it is also true that a model with these characteristics loses value if it is not accompanied by respiratory dysfunction, that is, if the entire process under study is not based on "clinical" changes. We presume that they certainly do exist, and we would like to verify this in future publications. The article also fails to demonstrate that the reaction is a typical response by T helper-2 lymphocytes with increased expression of cytokines such as interleukin-4, 5 or 13, and/or the presence of immunoglobulins E specific to ovalbumin. Meanwhile, there has been debate over whether or not it is appropriate to use, as the authors have done, adjuvants such as aluminium hydroxide, over whether it is fitting to sensitise by the intraperitoneal route rather than the intranasal route, and over the advantages and disadvantages of using ovalbumin instead of authentic airborne allergens (such as dust mites), etc. Ovalbumin does not cause asthma in humans. Can its use be justified by the fact that mice have specific T-cell receptors for ovalbumin? Certainly, a long-term approach should include creating similar mice whose T lymphocytes recognise airborne allergens.

Conclusions

Perhaps these comments really stem from impatience and our wish to have a better in-depth understanding of what happens in allergic asthma and how it happens, with the aid of this model and others. On the other hand, some of the ideas that are circulating, and may seem to constitute criticism, could reflect this study's greatest strength: what it presents urges us to learn more. Is this not the essence of research? Some of these data will surely be presented in the near future. It is therefore appropriate to develop the model. Its points of particular interest are the intensity of the inducement process and the magnitude of the response that it produces in the context of preclinical pharmacological studies of modulator (inhibitor) molecules. In this respect, we know that the differences observed are often on the very limit of statistical significance. One solution would be to increase the number of animals (the "n"), which gives rise to monetary, ethical and even workload limitations; another would be to develop a model with a more detailed pathological process which would be more susceptible to "statistically visible" modulation. And this study is on the right path.

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