

Survey of immuno-allergological ultra high dilution research

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Background: Experiments about basic research in Immuno-allergology reported by M. Bastide and B. Poitevin in *Ultra High Dilution* (1994) have been appraised from a 20 year perspective. The numerous experiments published mainly focus on immunological regulation, inflammatory process and basophil activation. They are analyzed according to one essential criterion: repeatability.

Methods: The commentary reflects the research details made available in a recently published literature review, also published in French.

Results: The regulatory effect of high dilution of bursin on immune response has been observed in multiple experiments but not reproduced by independent teams. The immunomodulating effect of Thymulin has been confirmed in mice. *Rhus toxicodendron* has an anti-inflammatory activity on different models, from mother tincture (TM) to very high dilutions. The homeopathic complex Canova activates macrophages *in vitro* and *in vivo*, induces lymphocyte proliferation, and reduces the size of tumors and mortality of sarcoma-bearing mice. Some homeopathic medicines used in clinical inflammation modulate *in vitro* the neutrophil activation, with variability in the protocols used and in the medicines tested. In allergology, high dilution histamine has an inhibitory effect on basophil activation in multicenter trials and with independent teams, either with methods implying a change in basophil staining or with flow cytometry. However, high dilution histamine had no effect in some well-conducted experiments. The inhibitory effect of *Apis mellifica* has not been studied with the flow cytometry method, as well as the activation of basophil by anti-IgE high dilution, published in Nature.

Conclusions: Despite considerable research activity in immuno-allergology and a great increase in the number of publications, there is still not in this domain a “gold standard” trial in basic research in homeopathy. The most studied system, the inhibitory effect of histamine high dilutions on basophil activation, requires clarifications of various factors, including individual sensitivity. For scientific and epistemological reasons, the same work should be carried out for independent reproduction of the experiments conducted with anti-IgE and *Apis mel* high dilution, in complement of the new axes of research in immunoallergology developed since 20 years. *Homeopathy* (2015) ■, 1–8.

Keywords: *Rhus toxicodendron*; Histamine; *Apis mellifica*; Anti-IgE high dilution; Memory of water

Introduction

Immunoallergology is a domain in which homeopathic basic research has been very productive^{1,2}, probably

because immunology and homeopathy share theoretical bases such as the “logic of the similarity” and “the principle of regulating endogenous systems of healing, the best known of which is certainly the immune system and its neuroendocrine integrations”³. In a general review, some specialists of homeopathic basic research put the emphasis on the importance of the repeatability of experiments⁴. In this perspective, we describe the new studies carried out in immunology and allergology, mainly those for which the repeatability criterion has been critical.

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Recent studies in immunology and inflammation

Immunomodulating effect of high dilutions

In the 80's, Bastide's group showed the immunomodulatory effect in mice of endogenous compounds such as thymic hormone from 4 to 11c, interferon, and interleukin 2.⁵ Moreover, high dilutions of bursin (equivalent to 13c or 14c) were able to regulate the immune response of chickens which had undergone bursectomy, by partially restoring the humoral immunity⁶, the pituitary-adrenal⁷ and the pineal activities⁸. In these experiments carried over 6 years⁹, the high dilutions of bursin seem to be able to act as substitute to the bourse of Fabricius to educate the B Lymphocytes.

Recently, the Bonamin's team¹⁰ evaluated the development of broiler chickens treated with Thymulin 5c by conventional zoo technical indices, phytohemagglutinin induced inflammation test and histomorphometric analysis of lymphoid organs (thymus, Fabricius bursa and spleen). Thymulin 5c, diluted in the water from the 1st to the 42nd day of life, increase the productivity index, associated with higher viability in the 7th week and a possible shunt to B lymphocyte activity. Thus, Thymulin 5c could improve productivity in poultry production due to its immune modulation properties. Thymulin 5c treatment also improves the granuloma inflammatory process induced by BCG in mice, by modulating local and systemic phagocyte differentiation, as well as T cell migration into the local lymph node¹¹.

In a similar model, Thymulin 5c improved B1 cell activation and *Leishmania amazonensis* phagocytosis efficiency in mice¹². This modulatory effect on Inflammation response to murine cutaneous Leishmaniasis differs from the effect of *Antimonium crudum* 30c¹³. In mice, Thymulin 5c have similar immunomodulatory effects as a biotherapy 30c containing active virus Influenza, improving the innate immune response¹⁴.

Effects of an homeopathic complex medicine: Canova

Canova, a homeopathic complex medicine developed in Argentina, is now produced in Brazil. The final product contains *Aconitum napellus* 11x, *Arsenicum album* 19x, *Bryonia alba* 18x, *Lachesis muta* 18x and *Thuya* 19x¹⁵. Canova is described as an immunomodulator which activates macrophages *in vitro* and *in vivo*¹⁵. It activates mouse peritoneal macrophages, alters their oxidative metabolism, modifies their ultra structure (enlarged nucleus, more euchromatin than heterochromatin, spreading and projections), increases the endosomal/lysosomal system and decreases the TNF-alpha production but not the IL-2 and IFN-γ production. Canova is also active *in vitro* on the human blood monocyte differentiation¹⁵. When lymphocytes are stimulated by activated human macrophages treated with Canova, it indirectly induces lymphocyte proliferation¹⁶. Canova also activates *Cebus apella* macrophages *in vivo* and *in vitro*. The lymphocytes cultured in a supplemented medium with *C. apella* macrophages activated by

Canova treatment presented a higher number of proliferation cells, and the production of Interferon gamma and interleukin-5 cytokines is induced. Thus, Canova has potential as a new adjuvant therapy¹⁷. Canova reduces the size of tumors and decreases the mortality on sarcoma 180-bearing mice. It increases lymphocyte TCD4 in normal mice, B and NK lymphocyte in S 180-treated mice, and increases total number of lymphocytes in both groups. These results suggest that protection against experimental sarcoma in mice may reflect an enhanced immune response of the host¹⁸.

Canova also has a synergic action with biotherapy. This complex modulates experimental *L. amazonensis* infection, controls infection progression and limits dissemination¹⁵. In mice, Canova interacts with the benzimidazole to modify the natural course of infection by *Trypanosoma cruzi*¹⁹ (Table 1).

Anti-inflammatory effects of *Rhus toxicodendron*

The action of *Rhus toxicodendron* (*Rhus tox*), a homeopathic medicine used in various inflammatory reactions (rheumatology, dermatology), has been tested *in vivo* on animals, particularly in India and in Brazil. In these countries, the confirmation of industrialized, composed, homeopathic medicines is required and pre-clinical assays are applied to homeopathic medicines²⁰. *Rhus tox* has an inhibitory effect on the rat paw edema caused by carrageenan for dilutions 6, 12, 30 and 200 c, the 6c dynamization being the most effective. *Rhus tox* 6c is also effective on the histaminergic edema induced by dextran and on the test of erythema in mouse ears induced by Croton oil. It is without effect on the vascular permeability induced by histamine and the formation of gastric ulcers, but it is effective in preventing the incidence of acute gastric ulcers induced by stress. *Rhus tox* 6c is also effective on pain by inhibiting the algogenic process caused by acetic acid, and interferes with inflammatory processes involving prostaglandins, leukotrienes, histamine and other inflammatory mediators²⁰.

In carrageenan induced paw inflammation in rats²¹, in its crude form *Rhus tox* exerts anti-inflammatory effects after a single dose, and proinflammatory effects after multiple doses. *Rhus tox* has also an immunostimulatory effect in its crude form as well as in 6c and 30c dilutions in mice: activation of humoral and cellular response, stimulation of phagocytosis, oxidative metabolism and chemotaxis of polymorphonuclear neutrophils. The higher dilutions 200c and 1000c have only a humoral effect^{21,22}.

Table 1 Immunomodulatory effect of Canova

- Activation of mice peritoneal macrophages *in vitro* and of differentiation *in vitro* of human blood monocyte¹⁵.
- Induction of lymphocyte proliferation by human activated macrophages¹⁶.
- Induction of lymphocyte proliferation by activated *Cebus apella* macrophages¹⁷.
- Protection against experimental sarcoma in mice¹⁸.
- Synergic action with biotherapy in *Leishmania amazonensis* infection¹⁵, and with benzimidazole in *Trypanosoma cruzi* infection¹⁹.

Rhus tox, in its crude form and dilutions (3c, 6c 30c, 200c) protects rats from Complete Freund's adjuvant (CFA) induced inflammatory lesions (best effect for 6c), body weight changes (best effect for 30c), hematological alterations, particularly the level of C-reactive protein, (best effect for 200c equal to diclofenac), and radiological joint alterations. *Rhus tox* improves the pain scores (best effect for crude form)²³.

Higher dilutions of *Rhus tox* (1M, 10M, CM) exert similar effects as lower dilutions on arthritic lesions; it improves body weight gain as well as pain, and protects rats against CFA-induced hematological and radiological perturbations²⁴. *In vitro*, *Rhus toxicodendron* 4×, 30×, 30c and 200c induce chondrocyte dedifferentiation and inflammatory responses, such as COX-2 expression and PGE2 production in primary cultured mouse chondrocytes.²⁵ (Table 2).

Effect of other homeopathic medicines on inflammatory process

Silicea is widely used in homeopathy for the treatment of chronic inflammations and infections, and abscesses. The production of Platelet activating factor (PAF) by peritoneal macrophages stimulated *in vitro* with yeast extracts has been enhanced in mice after administration of *Silicea* 5c and 9c²⁶. Higher dilution 9c had paradoxically more effect than the lower 5c. The replication of this study, organized by the French Veterinary Academy²⁷ has not been possible. In an experimental animal model based on induced chronic wounds in mice, *Silicea* was used by a group in Israel²⁸; the holes in the ears of the silica-treated animals were significantly smaller and healed faster than those in the saline-treated animals. The therapeutic effect also progressively improved with increasing *Silicea* dilutions.

Other homeopathic medicines are effective in inflammatory models. *Causticum*, a complex pharmaceutical preparation used only in homeopathy, acts as an oedematogenic agent when it is administrated in high concentrations into the hind paw of rats. In high dilutions (6c, 12c, 30c, 200c), *Causticum* inhibits the inflammation pre-induced by carrageenan. Pre-treatment with *Causticum* is without effect on the formation of granulomatous tissue²⁹. *Arnica* 6c inhibits carrageenan-induced rat paw edema, reduces nystatin-induced edema, a model of chronic inflammation, and also blocks histamine-induced increase of permeability³⁰.

Belladonna and *Echinacea angustifolia*, when prepared in "accord of potencies" modulate the macrophage activity induced by experimental peritonitis, increase the polymorphonuclear cell migration and have a cytoprotective action on leukocytes³¹.

Table 2 Anti-inflammatory effects of *Rhus toxicodendron*

- Inhibitory effect on the rat paws edema caused by carrageenan and prevention of the incidence of acute gastric ulcers induced by stress²⁰.
- Immunostimulatory effect in mice²².
- Protection of rats from Complete Freund's adjuvant induced inflammatory lesions^{23,24}.
- Induction of chondrocyte dedifferentiation and inflammatory responses²⁵.

In a model of itching induced in Wistar rats housed in a heated environment, *Dolichos pruriens*, in dilutions 9c, 12c and 30c, inhibits the itching, skin lesions, and fur thinning produced by heat³². But there was no effect from *D. pruriens* 6c to 30c, administered during 30 days to rats kept at usual temperature (20°C) (equivalent of pathogenetic effect). During the alveolitis process in rats, *Mercurius solubilis* 12c did not reduce bacterial growth, the microbiotica remained within the parameters of normality³³.

In the domain of isotherapy, the interaction of very high dilutions of dexamethasone 10⁻¹⁷ and 10⁻²³ M with usual concentration of dexamethasone has been studied on a model of acute inflammation induced by carrageenan in mice³⁴: the high dilutions partially blocked the anti-inflammatory effect of pharmacological dexamethasone with regard to path edema and polymorphonuclear cell migration.

Effect of homeopathic medicines on neutrophil activation

Polymorphonuclear neutrophils are particularly involved in the inflammatory process. In various experiments, these cells were the target of high dilutions of homeopathic medicines. The activation of neutrophils leads to enzyme secretion, generation of reactive oxygen metabolites, such as the superoxide radical O₂⁻ and hydrogen peroxide (H₂O₂), production of lipid mediators (chemotactic agent LTB₄) and of cytokines. The tissue damage caused by these mediators contributes to the long-term pathogenicity of allergic inflammation. *Belladonna* and *Ferrum phosphoricum* 5c and 9c had a significant inhibitory effect on the superoxide production by PMN activated either by zymosan or by formylated peptides (fMLP)³⁵. There were substantial differences in individual sensitivities to these homeopathic medicines. *Apis mellifica* (*Apis mel*) and *Silicea* had no effect. *Bryonia* and *Ferrum phosphoricum* were active on superoxide production of patients' blood with rheumatoid arthritis³⁶. The inhibitory effect of *Belladonna*, *Ferrum phosphoricum* and *Apis mel* 5c to 30c has been reproduced on the oxygen radical production of human PMN activated by fMLP or PMA³⁷. The stimulatory effect of *Bryonia* 4c and 9c on oxidative metabolism of polymorphonuclear leukocytes has also been reported³⁸.

Working on human neutrophils in culture and on neutrophil's oxidative metabolism, Bellative's team¹ observed an inhibitory effect of *Manganum Phosphoricum* 6x-8x, *Magnesia Phosphorica* 6x-8x, *Acidum citricum* 3x, *Acidum succinicum* 3x and 4x, and high dilutions of *Phosphorus* and *Magnesia Phosphorica*³⁹. The same team, using the superoxide dismutase inhibitable reduction of ferricytochrome c, observed an increase of superoxide production and an inhibition of adhesion with *Podophyllum* 4× and *Podophyllum compositum*⁴⁰.

Recent studies in experimental allergy

Between 1980 and 90, a series of experiments were performed on basophil activation^{1,41}. Three methods were used⁴²:

- changes in basophil staining with alcian blue⁴³ or toluidine blue⁴⁴, the basophil activation being accompanied by a loss of the staining affinity for basic dyes (achromasia),
- flow cytometric methods which determine changes in basophil surface markers after activation,
- measurement of histamine release.

In the early eighties, a similar model was used to study the modulation of basophil activation by high dilutions of histamine,⁴³ or by homeopathic medicines, *Apis mel* and Lung Histamine⁴¹. The third model used after 1987 in Benveniste' laboratory, direct activation by high dilution of anti-IgE, led to the most famous and controversial publication in homeopathy⁴⁵.

Among these three models, the inhibition of basophil activation by histamine, assessed in the 80s by the changes in basophil staining measured by the human basophil degranulation test (HBDT), and now by flow cytometry, has been described as a sensitive and reproducible model for the study of the biological activity of high dilutions. We present here a brief summary of the repeatability of these experiments, detailed in recent general reviews^{1,42,43}.

Effect of histamine high dilutions

Sainte-Laudy and Belon used a classical pharmacological model to study the biological effect of high dilutions: the negative feedback induced by histamine on basophils and mast cells activation via an H2 receptor. Using the HBDT, they observed the biological effect of high dilutions of histamine beyond the Avogadro limit on the achromasia of human basophils activated by an allergen extract (house dust mite), then by an anti-IgE antibody and fMLP. Histamine dilutions of 6-7c and 16-18c inhibit the degranulation of basophils induced by Dermatophagoides extracts⁴⁶. Dilutions of histidine (the carboxylated precursor of histamine) had no effect, confirming the specificity of the high dilutions⁴⁷. This effect of histamine was reversed by cimetidine, an antagonist of histamine H2 receptors. The enzyme histaminase inhibited the effect of ponderal doses (6c and 7c) but not that of high dilutions (18c), indicating that the latter was not due to the histamine molecule.

The activation of basophils by anti-IgE was significantly inhibited by histamine dilutions ranging from 15c to 18c⁴⁸ and by histamine 10^{-16} , 10^{-18} , 10^{-20} , 10^{-22} and 10^{-36} M⁴⁹. This effect was observed on basophils stimulated by small doses of anti-IgE and was not correlated with histamine release. In a multi-centre European trial performed in four laboratories, histamine dilutions 15c to 19c had a small but statistically significant inhibitory effect on basophil activation caused by the lowest concentration of anti-IgE, in 3 of the 4 laboratories. When all the data were combined together, there was a statistically significant inhibitory effect of the histamine containing solutions⁵⁰.

In the mid-1990s the same team tried to replicate the study using flow cytometry testing, which provides automatic control and observer independence compared with the manual basophil count necessary for HBDT. Two baso-

phil activation markers were used: CD63 and CD203c. The authors also used a mouse basophil model, in which histamine was measured spectrofluorimetrically. Using flow cytometry, Sainte-Laudy and Belon found an inhibition of CD63 expression after incubation with histamine doses of between 10^{-2} and 10^{-4} M and also with ultra-low doses of histamine 10^{-22} and 10^{-34} M⁵¹. In a further study, the same team observed the inhibitory effect of histamine 10^{-30} , 10^{-32} , 10^{-34} M, which was reversed by cimetidine, thus confirming the specific participation of the receptor to the action of ultralow dilution⁵².

An independent team⁵³ has observed a significant inhibition at 10^{-2} , 10^{-4} , 10^{-6} , 10^{-14} , 10^{-18} , 10^{-20} , 10^{-26} M histamine. This inhibitory effect was modified by the heating (70°C) of histamine dilutions but not by the freezing at -70°C. Another group⁵⁴, observed an inhibitory effect of high dilutions of histamine prepared in "good brandy", (recommended by Hahnemann as a diluent in 1827) and found that the diluents may influence the effect of high dilutions. In November 2002, the inhibitory effect of high dilutions of histamine was not found in a study organized by the BBC tv series Horizon. Some homeopaths took part in this study and others, including the author of this text, refused⁵⁵ for technical reasons: number of histamine dilutions tested, origin and numbers of donors, numbers of basophil used and of repetition of experiments⁵⁶.

According to Sainte-Laudy⁴³, the inhibitory effect of histamine high dilutions, in the range of 15–17c, is a reproducible phenomenon which disappears when histamine is replaced by histidine or in the presence of cimetidine. This phenomenon has been confirmed in a multicenter trial⁵⁷ using the HBDT model and by at least three independent laboratories using flow cytometry, including the Ennis group and the Italian group which confirmed, using a cytometric protocol with CD203c as an activation marker, the effect of histamine high dilution⁵⁸ (Chirumbolo *et al*, 2009). The Wiegant' team didn't observe significant variations⁵⁷. Using the mouse model, Sainte-Laudy and Belon observed that histamine high dilutions 15 and 16 c inhibit histamine release⁴³.

However, using flow cytometry, a Swiss group did not confirm the effects of high histamine dilutions on basophil function⁵⁹. In this study, basophils from the same human donor were incubated with diluted histamine (up to 10^{-34} M) or water controls and activated with anti-IgE antibodies in seven independent experiments. Histamine at the dilutions 10^{-2} M and 10^{-22} M was associated with a significant inhibition of basophil degranulation of 23.1 and 5.7%, respectively, if compared with control. However, if all controls were pooled, only histamine 10^{-2} M had a significant effect. A plate effect was discussed, but, according to the authors, was eliminated by randomization.

The preparation of leukocytes by dextran sedimentation, which modifies membrane properties of basophils, may explain the weak magnitude of inhibition observed even with high concentrations of histamine⁴³. However, a recent study of the same group, conducted in cooperation with Sainte-Laudy failed to reproduce the inhibitory effect⁶⁰. Guggisberg gives a summary of previously published

data using flow cytometric methods to determine the inhibitory effects of histamine, demonstrating that the histamine dilutions causing significant inhibition vary between the reported studies. This may be due to the susceptibility of basophils from different donors to histamine.

In conclusion, this experimental model, using the highly sensitive basophil activation phenomenon and the well-known negative feedback effect of histamine, has given positive results and can be considered as credible⁴³. However, Ennis in “a sceptical view”⁴² notes that, while there appears to be some evidence for an effect with the high dilutions in several different laboratories using the flow cytometric methodologies, the procedures of the laboratories have been poorly standardized. If a multi-centre trial is required, she suggests that before starting the trial, various points should be clarified, including the use of one cell donor or many, the use of multiple donors for experiments or repeat using just one donor, the number of anti-IgE dilutions, the determination of the best agonist, the method to detect activation and also the use of systematic negative control experiments to assess the stability of the experimental system. This set of approaches may help to solve what seems to be a “never-ending story”⁴².

Activation of degranulation of human basophils by anti-IgE high dilution

By their conception and implementation¹ these studies on the inhibition of basophil activation by homeopathic medicines and histamine differ from studies on direct induction by high potencies of anti-IgE antiserum, published in Nature⁴⁵. This multicenter study, led by J. Benveniste and involving four other laboratories, reported human basophils metachromasia not only at usual anti-

IgE antibody concentrations, but also at extremely high dilutions (10^{-60} or 10^{-120}). A pseudosinusoidal curve was observed when the human basophils were stimulated by increasing dilutions of anti-IgE up to dilutions of 1×10^{-120} . The results obtained were judged controversial by Nature, after they had accepted the publication⁶¹. These results were reproduced by an experienced investigator in the same laboratory⁶², but two others independent teams were unable to reproduce these findings^{63,64}. Objectively, there were problems of reproducibility for these studies on the direct activation of human basophils by high dilution of anti-IgE, even when the experiments were done in Benveniste's laboratory, and even when Davenas was the investigator⁴¹.

However some positive results have been observed, in very well controlled experiments. So, are the results of these experiments, which are difficult to reproduce, false? Only the use of new methods, including flow cytometry or non-subjective measurement of basophil achromasia can answer this question. The determining factors for the reproducibility of these results are the individual sensitivity of the different blood tested, the experimenter-dependence of the studies and the environmental factors capable of influencing the results⁴¹.

Inhibition of the degranulation of human basophils by *Apis mellifica* and lung histamine

Contrary to the above studies on histamine dilutions, the repeatability of the experiment relative to the inhibitory effect of homeopathic medicines, mainly *Apis mel*, has been less studied. These experiments were conducted from 1983 to 1991 in the Benveniste laboratory where HBDT was used for the biological detection of clinical allergy⁴⁴. We

Table 3 Repeated fundamental research studies for basophils model into homeopathically prepared dilutions 10^{-23} . In this table inspired from Endler *et al.*⁴ (Endler *et al.* 2010), we report the results obtained with very high dilutions on basophil activation, according to the method used and to the model (pharmacological regulation by histamine or homeopathic medicines, direct activation by anti-IgE). Only the main experiments are mentioned. ND: not done

Model	Method	Worksgroup	1: Initial study	2: Repetition Comparable effect	3: Repetition Different effect	4: Repetition 0 effect
Basophil & Histamine	Staining studies	Primary	Sainte-Laudy, 1991	Cherruault, 1989 Sainte-Laudy & Belon, 1991, 1993 Belon, 1999		
	Flow cytometry	Multicenter independent				
		Primary	Sainte-Laudy & Belon, 1996	Sainte-Laudy & Belon, 1997 Belon, 2004		
		Multicenter Independent		Brown & Ennis, 2001 Chirumbolo, 2009 Benveniste, 1991	Lorenz, 2003	Wiegant (Belon, 2004) Guggisberg, 2009 Wächli, 2012
Basophil & anti-IgE high dilutions	Staining studies	Primary Multicenter Independent	Davenas, 1998			Ovelgonne, 1992 Hirst, 1993
Basophil & <i>Apis mellifica</i> and Lung Histamine	Flow cytometry	ND				
	Staining studies	Primary	Poitevin, 1986	Poitevin, 1998 Benveniste, 1991		
	Flow cytometry	Multicenter Independent ND				

observed the inhibitory effect of *Apis mel* 9c and 15c on degranulation induced by allergens different the bee hymenopter venom,^{65,66}. *Apis mel* was chosen for its clinical indication and for the regulatory effect of phospholipase A2. The French Academy of Medicine requested the independent replication of these experiments. Some negative results were obtained by a team using granules diluted on basophils⁶⁷. However, two other teams (Paris, Marseille) reproduced the results, but didn't publish them, after the *Nature*' controversy in 1988⁵⁶. In the same year a new study was published on basophils activated by high and low doses of anti-IgE: basophil degranulation induced by low doses of anti-IgE antibody was strongly inhibited by high dilutions of Lung his and *Apis mel* with an inhibition of nearly 100% with Lung histamine 18c and *Apis mel* 10c⁶⁸. In a control study organized with an independent team of statisticians from INSERM, this significant inhibition of the achromasia by *Apis mel* was confirmed with four potencies, (15c, 16c, 17c and 20c) for the six potencies (15c to 20c) tested⁶². These results were observed by two investigators. It has not been possible since 1996 to develop a replication of this trial using flow cytometry⁴¹ (Table 3).

Recently, observing that diluted preparations obtained from *Apis mel* are reported in the homeopathic literature to have anti-inflammatory activity, the effect of *Apis mel* mother tincture (TM) and its 3c, 5c, 7c dynamized dilutions was explored on global gene expression profiles of human cells by means of microarrays using cluster analysis⁶⁹. Groups of genes up- or down-regulated with similar expression profiles among treatments were observed; other genes showed opposite regulation profiles at low and high dilutions of *Apis mel*. In particular, genes involved in cytokine expression, inflammatory processes, anti-oxidative responses and proteasome degradation were differentially, and sometimes divergently expressed by the TM or by *Apis mel* 3c, 5c and 7c dilutions.

Such a mechanism may explain the variation of effect of high and low dilutions of *Apis mel* on inhibition of basophil activation. Although *Apis mel*, a mixture prepared with entire honey bees crushed in a grinder and diluted in ethanol, seems to be an empirical medicine, its scientific exploration is still up-to-date.

Conclusion

In twenty years, there has been a considerable increase in the number of publications in homeopathic basic research: at the beginning of 2015, in the Homeopathic Basic Research Experiment data base⁷⁰ there were 2058 publications, and 276 in the field of immunology. There are now various axes of research in immunology, which is very positive for the development of homeopathic basic research in this field. We agree with the idea that “there is no a single “Nobel-prize”-experiment that could “prove” homeopathy”, and that the picture of homeopathy is “like a puzzle or like a mosaic... where all the little pieces are important for the realization of the whole image”². Some solid pieces can contribute to the creation of a beauti-

ful mosaic. The multiple experiments carried out with high dilutions of biological mediators like histamine and Thymulin, homeopathic remedies such as *Rhus tox*, or a homeopathic complex like Canova, reveal much information. However, considering the evolution of homeopathy basic research experiments in France, according to the authors⁷¹, “the dramatic decrease... in the mid nineties of the last century might be related to the spectacular publication of Benveniste's experiment of basophil degranulation in the high-ranked journal *Nature* in 1988 and its contentious aftermath”.

Perhaps part of the mosaic needs to be restored. From a scientific and epistemological point of view, it would be interesting to determine whether the biological results leading to the “memory of water” are wrong or not. Independent reproduction of the experiments conducted with anti-IgE and *Apis mel*, as published from 1986 to 1991, could help to improve the scientific image of homeopathy, in complement of the new axes of research in immunoallergology developed since 20 years.

Conflicts of interest

The author has no conflicts of interest.

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