



Immunological models in high dilution research following M Bastide

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In 1994, Madeleine Bastide described experimental models in immunology that were used during the 1980s to investigate high dilution effects on several biological systems. She classified the available papers in four categories: High dilutions of antigens; High dilutions of thymus, bursa and other hormones; High dilutions of cytokines; Immunopharmacological activity of silica. The studies about high dilutions of antigens were not continued after this period, but gave rise to a long process of a series of *in vitro* models on antigens and histamine dilutions, that led to the demonstration of the biological modulation effects of these preparations on basophil degranulation. During this process, a multi-centre study was performed, with a high degree of reproducibility among different independent laboratories.

The studies about high diluted cytokines, thymulin and other hormones opened a new line of scientific investigation, about the regulatory properties of endogenous substances prepared according to homeopathic methods. The most frequently studied substance, thymulin, when administered to mice at 5cH potency, is able to improve the activity of phagocytes in different experimental situations, such as viral, bacterial and parasitic infections. The immunopharmacological activity of silica was demonstrated, at that time, as an *in vivo* illustration of the homeopathic 'similia principle'. More recently, studies on silica have assumed another focus: the putative role of silica as active contaminant present in high dilutions. This paper presents a follow-up summary on these items, considering the evolution of discoveries from 1994 to 2014. *Homeopathy* (2015) 104, 263–268.

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Introduction

In her seminal 1994 review,¹ the late lamented Prof. Madeleine Bastide (Figure 1) showed that immunological models are useful for demonstrating the activity of homeopathic remedies or effects of high dilutions of hormones or mediators. The whole living organism is able to respond to homeopathic remedies as well as to antigens. Of course, the immunological response is not mechanistically comparable to the action of a homeopathic remedy, but the immune system is naturally stimulated by extremely low doses of antigens (as low as 0.1 ng [10^{-10} g]²). In an *in vitro* model, human monocytes have been shown to present optimal phagocytosis using 10^{-14} M postin, and

such activity was still evident at 10^{-18} M.³ Evaluations of the effects of high dilutions or very low doses in immunological models are still an interesting methodological possibility and open a new field of application using homeopathic preparations in both the therapeutic and agricultural fields.

Methods

The main sources of information for this review were personal contacts with members of the homeopathic research community, mainly during the annual symposium of the *Groupe International de Recherche sur l'Infinitésimal* (GIRI; <http://www.giriweb.com>; accessed May 21, 2015), and the MEDLINE and HOMBREX databases. Experimental studies that used animal models were extracted from the publications and grouped into models. No clinical or human-based research data were considered in this review.

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Figure 1 Prof. Madeleine Bastide (1935–2007). For bibliography, see Ref. 52.

Results

High dilutions of antigens

The original discussion concentrated on the work of Weisman and colleagues, 1992,⁴ in which high dilutions (10^{-15} – 10^{-33} M) of KLH protein (keyhole-limpet-hemocyanin) that are prepared in centesimal potencies, including those above Avogadro's number, were able to signal the immune system similarly to the antigen at measurable concentrations and induce the production of polyclonal immunoglobulin M (IgM). After a new immunization, a specific potency-dependent increase in monoclonal anti-KLH immunoglobulin G (IgG; an immune memory antibody) could be seen.

After this period, new articles on immune stimulation with highly diluted substances focused on allergy research. In 2000, Aabel and colleagues^{5,6} discussed the curative and prophylactic efficacy of *Betula* 30C in clinical trials. No prophylactic effect was found, but a curative effect was observed. The benefits of homeopathy's curative approach for treating allergies were reinforced by other authors a few years later.^{7–9}

Most studies that utilized this model discussed the modulation of *in vitro* human basophil degranulation by highly diluted antigens, such as *Apis mellifica*¹⁰ and histamine.¹¹ After 20 years of discussion, Sainte-Laudy and Belon published their final conclusion about the effectiveness of 15–17cH potencies of histaminum as a reproducible biological effect, which was confirmed by a large multicenter study¹¹ and most independent authors.^{12,13} The effect of highly diluted/succussed histamine presented high specificity. The same potencies of related nonspecific substances, such as histidine, had no effect and the histamine action was blocked by the H₂ receptor inhibitor cimetidine. The authors also discussed the putative role of histamine H₂ receptors in the mechanism of action.¹⁴ The reported difficulties of reproducibility in different laboratories suggest that this model may serve

to identify possible technical factors in the manifestation of the regulating power of high dilutions of histamine.

High dilutions of thymulin, bursin, and other hormones

Bastide, in 1994¹ discussed her own work on high dilutions of thymulin and bursin. Her first experiment was mentioned, in which C57/BL6 mice with P815 mastocytoma were treated with different centesimal potencies of thymulin and exhibited a clear immune depressive effect, with decrease in the specific anti-tumor activity of T cells.^{15,16} Another series of experiments that were performed by^{17,18} were also mentioned, showing that thymulin 9C modulated the T-cell dependent humoral antibody response and respective corticosterone serum levels in mice, with an emphasis on seasonal variations in these effects.

Another hormone that was studied by Bastide was bursin. In a sophisticated experimental model that was developed by Guellati, in 1990¹⁹ and Youbicier-Simo and colleagues, in 1993,²⁰ chicken embryos were surgically deprived of *Fabricius bursa* on the day 4 of incubation, and their specific B cell response recovered after *in ovo* treatment with different dilutions of bursin up to 10^{-40} M. The IgM and IgG titrations were two-times those seen in controls. This study demonstrated how patterns of biological functions can be programmed by the transfer of information from high dilutions to living systems and how developing tissues are sensitive to this.

New studies on homeopathic preparations of hormones were continued into the next decade. In 2001, Bonamin and colleagues²¹ performed a study that demonstrated the capacity of dexamethasone 7 and 15cH (10^{-17} and 10^{-33} M, respectively) to negate the anti-inflammatory effect of pharmacological concentrations of the same hormone using a classic experimental model of inflammation in mice. Some years later, effects on inflammation were also shown in adult rats that were born from mothers that were treated with dexamethasone 15cH during pregnancy. This was the first demonstration in rats of the mother-newborn transmission of homeopathic information and its ability to imprint functional 'patterns' on the physiology of offspring during the perinatal period.²²

Considering the effects of homeopathic thymulin, new experiments were subsequently performed after Bastide's publications. Sato and colleagues, in 2012,²³ showed the usefulness of thymulin 5cH diluted in drinking water (20 ml of thymulin 5cH in 50,000 ml of water) to improve weight gain, viability (from 75% to 97.5%), the cellular immune response against viruses (22%), and quality of life of broiler chickens (reflected by the productivity index; 22%) compared with controls, suggesting the use of homeopathic thymulin as a useful zootechnical tool, as a promoter of animal welfare.

In addition to the known improvement of the T-cell response that was described in mice and chickens, a more recent study reported the effects of thymulin 5cH on the recruitment of B1 cells (CD19+, CD11b+, and CD23– stem cells) from the peritoneum to local

granuloma lesions that were induced by BCG in a murine model. Under the effects of thymulin 5cH, migrating B1 cells were able to increase their differentiation rate in phagocytes at the site of chronic injury, thus improving the local innate immune response to reduce infection by approximately 22%.²⁴ A similar effect was seen in mice that were immunized with viral antigens, in which a 50% increase in B1 cells was found in the peritoneal cavity in treated mice after they were injected with the viral antigen.²⁵ Moreover, in an experimental model of *Leishmania (L.) amazonensis* infection in mice, B1 cell and phagocyte counts in the spleen increased by approximately 65% and 52% compared with controls, respectively.²⁶ A general view of the effects of thymulin is presented in Table 1.

High dilutions of cytokines

Bastide described a series of experiments, in which the recovery of normal T-cell function in NZB mice was seen after treatment with interferon $\alpha\beta$ at concentrations that were equal to 4-9C potency.²⁷⁻²⁹ NZB mice are known to present early thymus involution that impairs proper cellular immune function. The same dilutions also decreased macrophage activity in adult mice and increased this activity in pubertal mice.³⁰

Other studies were then reported in.¹ High dilutions of interleukin-2 (IL-2; 4-9C) were injected after repeated immunization and decreased antibody production,²⁹ and high dilutions of cytostatic substances stimulated macrophages and lymphocytes.³¹

More recently, a series of studies on the effects of highly diluted tinctures on cytokine production have been performed using modern molecular techniques, with the aim of developing new medicines to treat several diseases, such as cancer and infections. Interesting *in vitro* results were reported by,³² in which J774.G8 macrophages increased tumor necrosis factor- α (TNF- α) production after stimulation with *Influenzinum*. Ramachandran and colleagues, in 2007,³³ found that two commercial homeopathic products that are indicated for the treatment of influenza (Phase 6[®] and Flu Terminator[®]; Be Well Homeopathics, Miami, FL, USA) stimulated the production of

pro- and anti-inflammatory cytokines by human leukocytes.

Oliveira and colleagues, in 2011^{34,35} performed a wide screening of different homeopathic complexes that are able to modulate macrophage and lymphocyte function using *in vitro* and *in vivo* models and found that cytokine production can be a sensitive marker of their therapeutic potential. Some years previously, the same authors reported that the treatment of macrophages with Canova[®] (a homeopathic complex that is composed of *Aconitum*, *Thuya*, *Bryonia*, *Arsenicum*, and *Lachesis*) *in vitro* increased inducible nitric oxide synthase production, decreased TNF- α , IL-2, and IL-4 production, and regulated the expression of 147 genes that are involved in cell signaling and activation.³⁶⁻³⁸ In fact, evidence of the modulation of *in vitro* signaling processes between macrophages and lymphocytes was seen in lymphocytes that were able to produce interferon- γ and IL-5 when in co-culture with Canova[®]-activated macrophages.^{39,40}

Many other studies have indicated the sensitivity of cytokines to high dilutions in living systems at the cellular level.^{33,41-44} A large review of homeopathy and experimental immunology was published by Bellavite and colleagues, in 2006.⁴⁵

Immunopharmacological activity of silica

Two studies were discussed by Bastide. In one study, highly diluted silica potency-dependently modulated macrophages *in vitro*.⁴⁶ In the other study, different potencies of silica (up to 200C) increased the speed of wound healing in mice *in vivo*.⁴⁷ In addition to demonstrating the action of homeopathic medicines on macrophage activity, these studies were classic experimental demonstrations of the similia principle.

Unfortunately, although several studies on the modulation of macrophage activity by homeopathic medicines were performed during the following years,^{34,35,37-40,48} no study was performed that used silica as the stimulation agent, despite the fact that 'silica theory' is still under discussion. This theory is based on the hypothesis that silica nanoparticles that are present

Table 1 Biological effects of homeopathic thymulin*

| Thymulin potency | Effect | References |
|--|---|--|
| 9C (10 ⁻³ –10 ⁻¹¹ ng) | Reduction of T cell response against tumor in mice | Bastide <i>et al.</i> , 1985 ¹⁵ Bastide <i>et al.</i> , 1987 ¹⁶ |
| 9C | Effects of seasonal variations on humoral immune response in mice | Doucet-Jaboeuf <i>et al.</i> , 1984 ¹⁸ |
| 5cH diluted in drinking water (20 L) | Increase in spleen and bursa reactivity and reduction of broiler mortality at 40 days of life (from 25% to 2.5%) | Sato <i>et al.</i> , 2012 ²³ |
| 5cH diluted in drinking water (0.1 ml/250 ml) | Increase in peritoneal B1 differentiation into phagocytes and reduction of BCG infection (22%) | Bonamin <i>et al.</i> , 2013 ²⁴ |
| 5cH diluted in drinking water (0.25 ml/250 ml) | Doubling of B peritoneal cells after viral antigen challenge | Siqueira <i>et al.</i> , 2012 ²⁵ |
| 5cH diluted in drinking water (0.1 ml/250 ml) | 65% more B1 cells in the spleen in mice infected with <i>Leishmania (L.) amazonensis</i> , with a 30% reduction of local parasite infection | Santana <i>et al.</i> , 2014 ²⁶ |

* The potency is described according to the original citation.

during homeopathic medicine fabrication act as a carrier of homeopathic information between the mother tincture (i.e., the matrix of information) and living body (i.e., the receiver of information).^{49–51}

Discussion

Madeleine Bastide left a legacy of several contributions to immunology, toxicology, and biosemiotics^{52–54}. Her pioneering development of immunological models to study the effects and mechanisms of high dilutions of homeopathic medicines in the 1980s was her most important contribution. Based on the models that she, her co-workers, and her students created, a wide variety of new studies could be performed in subsequent years.

Based on the effects of highly diluted antigens and their specificity in the induction of immunological memory, the subsequent studies pointed toward the clinical usefulness of homeopathic approaches to the treatment of allergies and demonstrated the *in vitro* reproducibility of the effects of highly diluted substances. For instance, a similar basophil inhibition was reported also by high dilutions of adrenaline.⁵⁵ However, other authors could not reproduce the phenomenon in their laboratory.^{56,57} This process lasted 20 years and allowed the initiation of a new phase in homeopathy science, namely the search for the mechanism of action beyond simply the biological plausibility of such effects. For instance, recently, some studies about *Arnica montana* 6cH in animal models revealed that the mechanism involved is linked to vessel dynamics, improving lymphatic absorption of the inflammatory edema in an individual-dependent manner.^{58–60}

Hormones that are able to modulate the immune response continue to be an important source for new studies on such substances as corticosteroids, thymulin, and bursin. Further thymulin results have shown that homeopathic preparations of this hormone can be a useful for improving the quality of life of infected and stressed animals by modulating the innate immune process, with a focus on macrophage differentiation and function after infectious injuries. The interest to this hormone rapidly grew in recent years in literature: a substantial body of information pointed to thymulin as anti-inflammatory peptide with a number of regulating actions in the neuroendocrine axis.⁶¹ Studies on dexamethasone demonstrate the ability of high dilutions to 'impair' the effects of pharmacological concentrations of the same hormone and 'imprint' specific patterns of functionality on immune cells when they are exposed to the hormone during development.

Studies on cytokines in homeopathic models have led to another point or view that is different from their starting point, in which highly diluted cytokines themselves are used as immune stimulators. Many studies that were published after 1994 were developed with the purpose of discovering new and less toxic drugs that are able to treat cancer and infections. To achieve this, the most explored models were *in vitro* macrophage cultures and macrophage/lymphocyte co-cultures that were exposed to different homeopathic substances to establish a cytokine

panel of the supernatant that is able to mirror the patterns of functionality and properties of these cells.

With regard to the effects of highly diluted silica on macrophages, as seen in the pioneering studies, no new information was found that could be considered a continuation of this line of investigation. However, silica remains a focus of high dilution research because of its putative role as a carrier of homeopathic information after the still controversial 'silica theory' was proposed.

Conclusion

The immunological tools that are used to study homeopathy and highly diluted preparations that were proposed in the original chapter by Bastide, 1994, are still useful for understanding their mechanisms of action. New and refined models were developed from 1994 to 2014 for this purpose.

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