A review on immunomodulatory response of homoeopathic medicines through cytokine induction as evidenced in in vivo and in vitro studies

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Abstract

Background: This review discusses the importance of inter-individual variations in Homoeopathy and role of immune modulation through cytokine induction behind these variations in symptoms. Objectives: To analyse the effects of homoeopathic drugs in modulation of cytokine synthesis to find the individualised immunological mechanisms of these drugs. Methods: The PubMed database was searched for studies which analysed effects of homoeopathic medicines on cytokine synthesis. Full texts of shortlisted studies after scrutiny of abstracts, were analysed for study design, homoeopathic medicines used, cytokines analysed and results. Results: The PubMed search yielded 21 studies. After analysis of abstracts of 21 studies, 10 were shortlisted, which included 7 in vivo and 2 in vitro studies. One study had both in vivo and in vitro interventions. Majority of in vivo studies used rat and exposure to immunological challenge before administration of homoeopathic medicine. The cytokines studied were interleukin (IL)-1α, IL-1β, interferon-gamma, tumour necrosis factor alpha, IL-2, IL-4, IL-5, IL-6, IL-10 and IL-12. The two in vitro studies evaluated effects of Saussurea lappa and Mercurius solubilis on lymphocytes and macrophage culture, respectively. Out of 10 studies analysed, 8 showed homoeopathic medicines can modulate cytokine synthesis either by increasing or decreasing the cytokine synthesis, in a statistically significant manner. Conclusion: The studies were heterogeneous regarding the antigenic challenge given to stimulate immune cells and only 50% of studies clearly mentioned random allocation of animals in groups. Though majority of studies showed that homoeopathic medicines can modulate cytokine synthesis, the mechanism of cytokine modulation remained unexplored.

Keywords: Cytokines, Homoeopathy, Immunomodulation, In vitro studies, In vivo studies

INTRODUCTION

Importance of inter-individual variations in classical Homoeopathy

It is a common experience that patients, even when suffering from the same disease, do have some inter-individual variations in their symptoms. Although these variations are largely overlooked as insignificant by the conventional medicine, these were the principal reason behind Hahnemann’s objection to the use of disease terms as the primary goal of treatment. Hahnemann11 observed that clinical features could not be uniformly and specifically linked to diseases and are subjected to inter-individual variations (§73). The disease terms used in his time were symbolic of ideal cases of diseases, which do not admit any clinical variations and were far from being realistic (Footnote 71 of §73 in Organon [6th edition]). He even avoided the use of these terms in his classification of acute and chronic diseases. Besides Hahnemann, Sydenham31 also perceived the fallacy in formulating disease entity based on clinical features. These inter-individual variations in symptoms of patients are largely unexplored as to their mechanism and various terms such as ‘peculiar’, ‘strange’, ‘symptoms of the patient’, ‘symptoms that cannot be accounted for,’ etc. are used to describe them.

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Role of immunogenetic mechanisms in producing inter-individual variations in disease

Research over the past decade has revealed that the nature of clinical presentation in a disease is significantly determined by the host immunogenetic mechanisms. These mechanisms involve complex functioning of several cytokines and their interaction with various organ systems, especially the hypothalamic–pituitary–adrenal axis. The synthesis of cytokine molecules is governed by a cluster of genes which are located on short arm of chromosome 6 and are known as major histocompatibility complex (MHC). The alleles of these genes are unequally distributed between different racial and ethnic groups. The striking characteristic of these genes is that they show extensive allelic polymorphism so much so that every individual has a unique set of MHC gene alleles. Numerous studies have shown that the alleles of MHC genes that codes for cytokines are associated with several diseases including autoimmune diseases, immune defect disorders, infectious diseases, malignant conditions and even some non-immune conditions. These studies reveal that MHC heterogeneity is responsible for inter-individual variations in susceptibility/resistance to these diseases. The MHC heterogeneity is also reflected in the expression of various cytokines which are immune modulators. Recent reviews suggest that an individual’s genetically determined ability to induce changes in pro- and anti-inflammatory cytokines levels causes a wide variety of symptoms produced during acute and chronic illnesses. The polymorphism in MHC genes alleles contributes to the inter-individual variations in the production of various cytokines. These inter-individual variations in cytokine induction may explain the inter-individual variations in the severity of clinical illness and in propensity to develop complications. This fact is of considerable significance in context to classical Homoeopathy because it emphasises the important role played by host immune system, and cytokines in particular, in producing the individual specific symptoms.

What are cytokines?

Cytokines are a group of polypeptides secreted by lymphoid and non-lymphoid cells. These polypeptides serve a wide range of functions, but their principal function is modulation of immune response and intercellular communication. The characteristic features of cytokine action are:

1. Cytokines have multiple effects. They often have more than one type of receptors
2. Redundancy: Cytokines can be redundant. Their receptors often share subunits. Therefore, several different cytokines can mediate the same or similar function
3. Cytokines can have specific and unique functions
4. Cytokine synergism: The combined effect of multiple cytokines on cellular activity can be greater than the additive effects of individual cytokines
5. Cytokine antagonism: The effects of one cytokine can inhibit or offset the effects of another cytokine
6. Many properties of cytokines are shared by hormones and growth factors so the distinction between these three classes of mediators is often blurred
7. Cytokines induce local and constitutional symptoms. The key symptoms of inflammation including pain, swelling, redness and warmth are produced by pro-inflammatory cytokines released at the site of inflammation. The pro-inflammatory cytokines not only cause local manifestations of inflammation but also orchestrate a variety of behavioural, physiological, endocrine and neural changes produced during inflammation, injury and infection. These include fever, sleep disturbances, fatigue, anhedonia, depressed mood, decreased activity and social interaction, decreased food and water intake, formation of taste aversion to novel foods, etc. These constitutional symptoms are produced by cytokines through their action on hypothalamic–pituitary–adrenal axis. Indeed, many symptoms found in chronic illnesses such as cancer can be induced in animal models by administration of pro-inflammatory cytokines. Physical and psychosocial stressors can also modulate the release of pro-inflammatory cytokines.

We propose that similar cytokine driven immunomodulatory mechanisms also contribute to the individualised symptom picture derived during homeopathic drug proving and these mechanisms may supplement the drug proving records in the applicability of the Law of Similaris. The best experimental model to explore these immunologic mechanisms would be the in vitro and in vivo studies.

We conducted a review of studies which analysed the effects of homoeopathic drugs in modulation of cytokine induction to find the individualised immunologic mechanisms of homoeopathic medicines.

**Methods**

The PubMed database was searched for the concerned studies with following search parameters: The search term used was ‘Homeopathy AND cytokines’ with search details - (‘homeopathy’ [All Fields] OR ‘homeopathy’ [MeSH Terms] OR ‘homeopathy’ [All Fields]) AND (‘cytokines’ [MeSH Terms] OR ‘cytokines’ [All Fields]) AND ((‘1980/01/01’[PDAT]: ‘2020/03/31’[PDAT]) AND ‘animals’ [MeSH Terms]) and following filters: article types – clinical trial, clinical trial veterinary, controlled clinical trial, observational study veterinary, randomised controlled trial and systematic reviews; publication date – from 1st January, 1980 to 31st March, 2020; species – other animals. The search result was further narrowed on the basis of the following criteria:

a. The study should be in vivo or in vitro study
b. The study should test the effect of homeopathic drug, either individually or in combination, on induction of cytokines.

**Results**

The PubMed search result yielded 21 studies. Abstracts of the 21 studies were first analysed to filter those studies...
which analysed homoeopathic medicines for their immune modulation through cytokine induction. Total ten studies were selected on the basis of their abstracts. Out of ten studies, seven were in vivo studies[25-32] and two were in vitro study.[33,34] One study[29] had both in vivo and in vitro intervention.

Full text of these 10 studies were analysed for the following details:
A. Study design
B. Homoeopathic medicine used in studies
C. The cytokine studied
D. Outcome of the studies.

**In vivo studies**

**Study design**
The types of animal model used in in vivo studies were rats in five studies,[26,27,30-32] mice in one study,[28] and **Seriola rivoliana** (Longfin Yellowtail fish) in one study.[29] All in vivo studies had control groups. In six studies, the study animals were first exposed to any of the following immunological challenge such as: Exposure to pathogen (**T. cruzi-Y strain**, **V. parahaemolyticus**), induction of sepsis, blood induced inflammation or inoculation of prostate cancer MAT-LyLu cells. This was followed by administration of homoeopathic medicine. The control group received only immunological challenge.

In two studies,[25,28] homoeopathic medicines were tested before and after antigenic challenge. Mazon-Suastegui et al.[25] studied the effects of homoeopathic treatment prepared from **Vibrio parahemolyticus** and **Vibrio algimolyticus** (H1) and commercial homoeopathic medication **Phosphoricum acidum** and **Silicea terra** (H2) on the immune and antioxidant response in **Seriola rivoliana** juveniles under usual culture conditions and when challenged with **Vibrio parahaemolyticus**. The immune response was studied through quantitative polymerase chain reaction (qPCR) analysis of changes in expression of genes related to cytokine interleukin (IL)-1β, besides other proteins. H1 treatment led to overexpression of the IL-1β genes in weaning and early juvenile stage of fish with respect to control, whereas the H2 treatment led to underexpression of the IL-1β genes in the early juvenile stage. In fish challenged with **V. parahaemolyticus**, both H1 and H2 led to overexpression of IL-1β genes. Mayer et al.[28] tested homoeopathic combination medicine **Engystol®** prior to and post-antigenic challenge in mouse model. Lymphocytes derived from spleen were cultured. Supernatants from culture were collected and screened with a 32-cytokine panel. Results showed that **Engystol** alone did not alter immunity; however, on vaccine challenge, **Engystol** altered select cytokines/chemokines.

**Homoeopathic medicine used in in vivo studies**
The homoeopathic medicines tested were **Lycopodium clavatum** 13c, **Phosphorus 13cH**, **Engystol®, Canova, Traumeel S**, **Mercurius solubilis**, a combined therapy of **Conium maculatum**, **Sabal serrulata**, **Thuja occidentalis**, **Asterias**, **Phytolacca** and **Carcinosin 1000 c** and a homoeopathic medication comprising **Phosphoricum acidum** and **Silicea terra**.

**The cytokine studied in in vivo studies**
The cytokine studied were IL-1α, IL-1β, interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), IL-2, IL-4, IL-5, IL-6, IL-10 and IL-12. The techniques used for analysing cytokine were enzyme-linked immunosorbent assay (ELISA),[30] magnetic bead-based assays,[27] 32-cytokine panel assay,[25] quantification of cytokine mRNA,[31] quantitative polymerase chain reaction analysis[25] to study changes in the expression of IL-1β cytokine gene and use of mouse-mouse hybridoma plasma cell line 7TD1.[32]

**Results of the studies**

In five in vivo studies,[25-27,30,32] the homoeopathic intervention influenced the cytokine induction in a statistically significant manner as compared to control groups. In the study[31] assessing effect of homoeopathic treatment on gene expression in Copenhagen rat tumour tissues using quantification of cytokine mRNA, no significant differences were observed for any of the cytokine’s mRNA analysed between Homoeopathy-treated and water-treated controls. In the study[28] which tested immunomodulatory effects of **Engystol®** in C57BL/6 mice, pre- and post-vaccine challenge using a 32-cytokine panel assay, the cytokine panel showed some numerical shifts (increase or decrease) in the means over time; however, none of these data were statistically significant.

**In vitro studies**
The in vitro study by Sarwar and Enbergs[33] assessed the effects of **Saussurea lappa** root extracts prepared in ethanol on mitogen-induced IFN-γ in the cultures of peripheral blood mononuclear cells of goats in vitro. Different doses (10 μl, 2 μl, 1 μl and 0.5 μl) of decimal dilutions (D4, D6 and D8) of **Saussurea lappa** in sterile 0.9% NaCl solution were used. The mononuclear cells were cultured in RPMI medium and were activated by mitogen phytohemagglutinin (2.5 μg/ml). The IFN-γ levels in cell culture supernatants were determined by ELISA. Lymphocyte cultures were carried out in triplicate in 96-well flat-bottomed micro-titre plates which were allocated to experimental and control groups. All experimental wells received homoeopathic drug dissolved in 0.9% NaCl, whereas all control wells received RPMI + and NaCl. Culture plates were incubated for 24 h at 37°C in a humidified atmosphere of 5% CO₂. IFN-γ levels in the supernatants were determined by ELISA kit.

Result of the study: The mitogen-induced (PHA, 2.5 μg/ml) secretion of IFN-γ in cultures of peripheral blood mononuclear cell supernatants was suppressed by **Saussurea lappa** in dose-dependent manner.

The study by de Oliveira et al.[34] evaluated action of highly diluted **Mercurius solubilis** (Merc sol) in activation or modulation of macrophage functions. In in vitro experiment, the effects of **Merc sol** in the 6, 12, 30 and 200 centesimal potencies were evaluated on mice peritoneal macrophages. The harvested peritoneal macrophages were plated on Dulbecco’s Modified Eagle’s Medium (DMEM) + in 96-well tissue culture plates. Two hours after plating, the cells were treated with **Merc sol**.
sol or control solution. After 48 h of culture, the production of cytokines TNF-α, IFN-γ, IL-2, IL-4 and IL-5 was measured in the culture supernatant through FACSCalibur flow cytometer. Result of the study showed that Merc sol induced IFN-γ production at lower dilutions (6 and 12 CH), whereas higher dilutions (200 CH) of Merc sol stimulated IL-4 production.

**Study with in vivo and in vitro intervention**

Moreira et al. [29] evaluated the effects of homoeopathic medicine Canova on healthy Cebus apella lymphocytes. Healthy Cebus apella were treated in homoeopathic and negative control groups by homoeopathic medicine Canova and normal saline, respectively. In the positive control group, Canova was added at 10% of total culture medium volume after 3 h of culture of macrophages collected from three C. apella with no in vivo treatment. The peritoneal macrophages and peripheral lymphocytes of Cebus apella were sequentially cultured in the same culture medium. During lymphocyte culture, the culture media were supplemented with 2% phytohemagglutinin which acted as mitogen and antigenic challenge and stimulated metabolic activity and cell division in lymphocytes. The supernatants of the lymphocyte cultures were harvested and IFN-γ and IL-5 in the supernatants were detected by capture ELISA. The positive control group and Canova groups, with macrophages activated by Canova ex vivo and in vivo, respectively, presented a significantly increase of IFN-γ (P = 0.02 and P < 0.001, respectively) and IL-5 (P < 0.001 and P < 0.001, respectively) levels than negative control group.

**Discussion**

We analysed all selected studies for randomisation and blinding in their methods. Only 50% of studies clearly mentioned random allocation of animals in groups and only three studies were performed blinded. The studies were heterogeneous regarding the antigenic challenge given to stimulate immune cells. In two in vivo studies [25,29] and one in vitro study [34], the effect of homoeopathic medicines prior to or without antigenic challenge was studied. This can be considered as a pure effect of homoeopathic medicines on modulation of cytokine synthesis. Out of these three studies, two studies [25,34] showed that homoeopathic medicines can modulate cytokine synthesis without antigenic challenge in a statistically significant manner. Total eight out of ten studies showed that homoeopathic medicines can modulate cytokine synthesis either by increasing or decreasing the cytokine synthesis in a statistically significant manner. However, the mechanism of cytokine modulation remained unexplored. One study [31] analysed the expression of genes involved in cytokine synthesis in tumour and lung tissues of rats, to explore possible mechanisms of action of homoeopathic medicines; however, no significant differences were observed for any of the cytokine’s mRNA analysed between Homoeopathy-treated and water-treated controls. In six studies [25–28,32] homoeopathic combination medicines were tested which included Canova, Engystol and Traumeel S. Four studies [26,27,33,34] tested individual medicine Lycopodium clavatum 13c, Phosphorus, Saussurea lappa and Mercurius solubilis. Two studies [31,34] tested homoeopathic medicines in high dilutions. Two studies [26,27] evaluated the immune modulatory effects of Lycopodium clavatum and Phosphorus on a time scale. Lycopodium clavatum modified the immune response with increase of cytokine IFN-γ on day 10 and IL-12 on day 24 and decrease of cytokine IL-10 concentration on day 10 and subsequent increase of this cytokine and IL-4 on day 24. The treatment with Phosphorus caused a significant increase of INF-γ and TNF-α on the 5th day of infection compared with the control (P < 0.05), with reestablishment on the 24th day.

Our review was limited to the search made only on one database. The result of the review suggests that more rigorous randomised controlled trials, with blinding, are required to replicate the results of the selected studies. The results of the studies discussed have limited implications in the sense that only selected cytokines were analysed and results obtained on animals or animal derived cells cannot be extrapolated on humans. Use of human hematopoietic cells in ex vivo cultures may be considered to overcome this limitation. There is also a need of better study models which can assess immunomodulatory effects of individual homoeopathic medicines at both local and systemic levels as well as study models which can explore molecular mechanism behind modulation of cytokine synthesis.

**Conclusion**

The studies were heterogeneous regarding the antigenic challenge given for stimulation of immune cells and only 50% of studies clearly mentioned random allocation of animals in groups. Modulation of cytokine synthesis was found to be statistically significant in eight out of ten studies however the mechanism of cytokine modulation remained unexplored.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

None declared.

**References**

4. Blackwell JM, Jamieson SE, Burgner D. IL-10 and subsequent increase of this cytokine and IL-4 on day 24. The treatment with Phosphorus caused a significant increase of INF-γ and TNF-α on the 5th day of infection compared with the control (P < 0.05), with reestablishment on the 24th day.

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Mathur and Kapoor: Immunomodulatory response of homoeopathic medicines


Un examen de la réponse immuno-modulatrice des médicaments homéopathiques par induction de cytokines comme le prouve les études In-Vivo et In-Vitro.

Contexte: Cette revue discute de l'importance des variations interindividuelles de l'homéopathie et du rôle de la modulation immunitaire par induction des cytokines derrière ces variations des symptômes. Objectifs: Analyser les effets des médicaments homéopathiques sur la modulation de la synthèse des cytokines pour trouver les mécanismes immunologiques individualisés de ces médicaments. Méthodes: La base de données de PubMed a été recherchée pour les études analysant les effets des médicaments homéopathiques sur la synthèse des cytokines. Les textes complets des études présélectionnées après examen des résumés ont été analysés pour la conception de l'étude, les médicaments homéopathiques utilisés, les cytokines analysées et les résultats. Résultats: La recherche PubMed a donné 21 études. Après l'analyse des résumés de 21 études, 10 ont été présélectionnées, dont 7 études In-Vivo et 2 études In-Vitro. Une étude avait les deux interventions In-Vivo et In-Vitro. La majorité des études In-Vivo ont utilisé des rats et une exposition à un défi immunologique avant l'administration de médicaments homéopathiques. Les cytokines étudiées étaient l'interleukine (IL) -1α, l'IL-1β, l'interféron-gamma, le facteur alpha, l'IL-2, l'IL-4, l'IL-5, l'IL-6, l'IL-10 et l'IL-12 de nécrose tumorale. Les deux études In-Vitro ont évalué les effets de Saussurealappa et Mercuriussolubilis sur les lymphocytes et la culture des macrophages, respectivement. Sur 10 études analysées, 8 ont montré que les médicaments homéopathiques peuvent moduler la synthèse des cytokines soit en augmentant soit en diminuant la synthèse des cytokines, d'une manière statistiquement significative. Conclusion: Les études étaient hétérogènes en ce qui concerne le défi antigénique dû pour stimuler les cellules immunitaires et seulement 50% des études ont clairement mentionné la répartition aléatoire des animaux dans les groupes. Bien que la majorité des études aient montré que les médicaments homéopathiques peuvent moduler la synthèse des cytokines, le mécanisme de modération des cytokines est resté inexploré.
Revisión de la respuesta inmunomoduladora de medicamentos homeopáticos a través de la inducción de citocinas, como se evidencia en los estudios in-vivo e in-vitro.

Antecedentes: Esta revisión discute la importancia de las variaciones interindividuales en la Homeopatía y el papel de la modulación inmune a través de la inducción de citocinas detrás de estas variaciones en los síntomas. Objetivos: Analizar los efectos de los medicamentos homeopáticos en la modulación de la síntesis de citocinas para encontrar los mecanismos inmunológicos individualizados de estos fármacos. Métodos: Se buscaron estudios en la base de datos PubMed que analizaban los efectos de los medicamentos homeopáticos en la síntesis de citocinas. Se analizaron textos completos de estudios preseleccionados tras el escrutinio de resúmenes, para el diseño del estudio, los medicamentos homeopáticos utilizados, las citocinas analizadas y los resultados. Resultados: La búsqueda de PubMed produjo 21 estudios. Tras el análisis de resúmenes de 21 estudios, se preseleccionaron 10, que incluyeron 7 estudios in vivo y 2 in vitro. Un estudio tuvo intervenciones tanto in vivo como in vitro. La mayoría de los estudios in vivo utilizaron ratas y exposición a desafíos inmunológicos antes de la administración de la medicina homeopática. Las citocinas estudiadas fueron interleucina (IL)-1α, IL-1β, interferón-gamma, factor de necrosis tumoral alfa, IL-2, IL-4, IL-5, IL-6, IL-10 e IL-12. Los dos estudios in vitro evaluaron los efectos de Saussurea lappa y Mercurius solubilis sobre los linfocitos y el cultivo de macrófagos, respectivamente. De los 10 estudios analizados, 8 mostraron que los medicamentos homeopáticos pueden modular la síntesis de citocinas aumentando o disminuyendo la síntesis de citocinas, de manera estadísticamente significativa. Conclusión: Los estudios fueron heterogéneos en cuanto al desafío antígeno que se dio para estimular las células inmunitarias y solo el 50% de los estudios mencionaron claramente la asignación aleatoria de animales en grupos. Aunque la mayoría de los estudios mostraron que los medicamentos homeopáticos pueden modular la síntesis de citocinas, el mecanismo de modulación de citocinas permaneció inexplorado.

Eine Überprüfung Auf Immuno-Modulatorische Antwort Homöopathischer Medikamente Durch Zytokin-Induktion, Wie gezeigt In In-Vivo Und In-Vitro-Studien.


回顾在顺势疗法医学免疫调节的反应通过细胞因子归纳作为见证的活体内和体外研。

背景：这项审查讨论了顺势病和免疫调节作用通过细胞因子诱导这些症状的变化背后的个体间变化的重要性。

客观：分析顺势疗法药物的作用在细胞因子综合的模块化发现这些药物被赋予个性的免疫学机制。方法：PubMed数据库被搜寻了在细胞因子综合分析顺势疗法医学的作用的研究。在审查摘要之后，对入围研究的全文进行了研究设计，使用顺势疗法药物，细胞因子分析和结。结果：PubMed查寻产生了21项研究。在对21项研究摘要的分析以后，10被列名了，其中包括7项体内研究和2项体外研究，一项研究进行了体内和体外干预。多数活体内研究在顺势疗法医学的管理之前使用了对免疫学挑战的鼠和暴露。研究的细胞因子是白细胞介素 (IL)-1 α, IL-1 β, 抗原素伽玛, 肿瘤坏死因子阿尔法, IL-2, IL-4, IL-5, IL-6, IL-10和IL12。两项体外研究分别评估Saussurealappa和Mercuriussolubilis对淋巴细胞和巨噬细胞培养的影响，分别。在分析的10项研究中，8显示顺势疗法药物可以通过增加或减少细胞因子合成来调节细胞因子合成，具有统计学意义。结论：这些研究对于刺激免疫细胞的抗原挑战是异质的，只有50%的研究明确提到随机分配动物组。虽然大多数研究表明顺势疗法药物可以调节细胞因子合成，但细胞因子调制的机制仍然未开发。