**VEGETAL ADJUVANTS: WHERE TO?**

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**Abstract**: Adjuvant research is a rapidly progressing field, reflecting the increased rate of identifying new compounds with stimulating activities and meantime, a better perception of the immune mechanisms, possibly due to the progress in immunobiology. A risk/benefit analysis in using novel adjuvants should include the severeness and prevalence of the disease that needs prevention. The use of standardized methods enhances an evaluation of adjuvant safety.

Vegetal extractions from various sources are more and more often used, showing favorable influence in diminishing the negative impact of numerous agents or in increasing the non-specific or specific resistance of the body to infections. Their use as adjuvants remains a still open question for research.

Key words in any study on the influence of certain vegetal extractions on the immune system and their adjuvant qualities should necessarily define active principles from the plants, the immune function and adjuvant activities. Beyond the arid terminology, the interrelation of these elements creates a closed molecular circuit. Biologically active molecules, starting from the „nature’s pharmacy”, pass through the filter of the immediate cellular immunity, than through that of the specific immunity and reach, by the pass of induced molecules, the final result which is augmentation/amplifying of the immune response and improved production of protective molecules – antibodies.

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Association of extractive preparations with those used in classical therapeutic protocols could intervene in the increase of non-specific protective capacity of the individuals, by their complex immune-stimulating effect. The problem is the most actual in veterinary medicine, where certain stress-induced changes could be corrected in this manner. Such conditions are more and more frequent under intensive and meanwhile more and more artificial breeding/exploitation of the domestic birds and mammals, connected to an often misbalanced and incorrect nutrition (Zarnea G., 1990; Mizoguchi Y. et al., 1987; Muntean S., 1990). Nevertheless, numerous extractions of various plants are currently being used in augmenting the natural resistance to infections or for their treatment. Moreover, active principles of plants serve to partially restore the immune reactivity in individuals with innate immune suppression (Bauer R., 2002; Benecia F. et al., 1995; Bezanger-Beauquesne L., 1993; Bussing A. et al., 1997; Candinas D. et al., 1996).

A less investigated field of the vegetal extractions’ use, but one with exquisite practical perspectives, is that of identification of novel adjuvants for vaccines, a stage considered to be essential in the development of modern vaccines (Vogel F.R., 2000;
Kaufman P.B. et al., 1999). Total vegetal extractions or extractive components from various plants could show such qualities. Thus, pertinent studies, showing a specific care for the detailed modulating mechanisms, tend to build a bridge between plant bioactive molecules and their sensitive receptor, the immune system.

One of the essential mechanisms of the immune (re)activity, the tolerance, represents a guarantee that the immune resources of the organism will not be lost in useless attacks directed towards its own (self) structures, but against pathogenic microorganisms. The result of the battle against the aggressors is the return to “order”, to the basic functional level, in other words, the restoration of immunological equilibrium. The activated effector cells should be removed at the end of their combat, before (re)orienting their activities against the individual’s tissues, as the production of activating cytokines ceases as well. This return to homeostasis is essential for the survival/existence of the organism.

Which are the possibilities to beneficially intervene for the host, when the protective capacity of the immune system is being blocked by the microbial aggressors?

The vast domain of infectious diseases’ is being governed at present by the prophylaxis of the nosological entity. Prevention, that also involves protection following vaccination and obtaining vaccines that generate maximal antibody synthesis, is a primary orientation in this field. There are several differences between the first vaccine obtained by Jenner in 1798 to stop the spreading of small pox and actual vaccines, obtained by genetic manipulations of the microbial agents. In improving the immunogenic capacity of the vaccines, an important step was taken by the use of adjuvants. Knowledge of the immunological mechanisms allowed defining the term “adjuvant”, as well as the understanding of various ways these compounds intervene to augment the immune response of the host. Thus, the perspective of active immunization encounters nowadays two main developmental directions: obtaining new antigenic units and discovering compounds with increased adjuvant efficacy and minimal side effects (Hilton L.S. et al., 2002).

The availability of the antigen is one of the most important aims in producing and developing the vaccine production, regardless of their type (Jiang Z.H., Koganty R.R., 2003). Emulsified adjuvants were favored for a long period of time, for their capacity to gradually release the antigen, prolonging its contact with the immune system. Alluminium hydroxide still represents the only adjuvant in use for authorized vaccines within the United States of America. By use of animal models, numerous types of compounds proved, over time, to be better adjuvants that the alluminium hydroxide, improving both cell-mediated and humoral immune responses. Nevertheless, their implementation in practice needs prolonged clinical studies (Vogel F.R., 2000).

Adjuvant research is a rapidly progressing field, reflecting the increased rate of identifying new compounds with stimulating activities and meantime, a better perception of the immune mechanisms, possible due to the progress in immunobiology. In turn, the adjuvants are being used in studies on basic immunology, such as antigen presentation to dendritic cells and modulation of the immune response through cytokines and their receptors. Adjuvants could be used with no doubt, in various immunization schemes, being useful in identifying the needs of protective immunity, different adjuvants inducing variations of the response to the same antigen.

The development of safe and effective vaccines built of antigenic subunits need a selective orientation of the protective immunity. Use of adjuvants that facilitate and direct the immune response to subunit antigens at increased levels is a critical component of rational vaccination protocols (Glenn G.M. et al., 2003; Goel V. et al., 2002). Adjuvants possess different action mechanisms and have to be selected based on the induced protective immune
response (in ex., dominance of antibodies, mucosal immunity, etc.). These compounds could improve the immune performances by directing the antigen to the antigen presenting cells (APC), by inducing cytokine synthesis to direct Th1 or Th2 cell mediated responses, and by promoting cell mediated immunity and reducing the number of shots or the amount of antigen needed for a protective immunization. Beneficial effects should be carefully measured, in comparison to risks of inducing undesirable local or systemic reactions. A risk/benefit analysis in using novel adjuvants should include the severeness and prevalence of the disease that needs prevention. The use of standardized methods enhances an evaluation of adjuvant safety.

Current trends in medicine tend to include natural products in therapy, without mixing allopathic and homeopathic treatments. Natural products gain more and more credit in comparison with chemically obtained ones. Within this framework, researches on the immune stimulating activities of vegetal extractions were successful, showing obvious immune modulating effects.

Briefly, the use of medicinal plants and their extractions as adjuvants for therapy, although known for centuries, is far from being a closed subject. Medicine and biochemistry show a continuously increasing interest to this field, resulting in the introduction in practice of novel preparations, gaining new meanings within modern prophylactic and therapeutic alternatives.

The triumph of modern vaccinology is being represented by defining and setting in practice of the vaccine concept. Vaccination as a procedure, represents the only method of immunological manipulation that benefited of a major success, taking the advantage of natural specificity of the immune effectors. Immunogenicity of the vaccine often depends on the adjuvants that enhance, directly or indirectly, antigen presenting cells’ (APC) activity, necessary to initiate the immune response. Purified molecules, and not only those, are not strongly immunogenic by themselves, thus, in their majority, non-cellular vaccines need adjuvants (Allison A.C., Byars N.E., 1986; Audibert F.M, Lise L.D., 1993; Vogel F.R., 2000), defined as compounds that enhance the immunogenicity (Ben Ahmeida E.T. et al., 1993; Jiang Z.H., Koganty R.R., 2003).

The adjuvant effects in therapy exerted by reputed medicinal plants were studied in complex experiments. These studies were connected especially to the investigation of anti-inflammatory activities (Chemli R. et al., 1990; ), changes in total leukocyte (Wagner H. et al., 1985) and macrophage numbers and populations (Percival S.S., 2000), micro- and macrophage respiratory burst (Dugenci S.K. et al., 2003).

Other experiments aimed to establish the action mode of fractions isolated from these plants: super antigen qualities that induce clonal selection of T lymphocytes (Delcourt M. et al., 1996; Saul F.A. et al., 2000), mitogenic activity for the same lymphocytes (Galelli A., Truffa-Bachi P., 1993; Le Moal M.A. et al., 1992a,b), interleukine synthesis (Le Moal M.A., Truffa-Bachi P., 1988). All these separate results offered, along with difficulties in interpretation, an incomplete overall picture of the stimulating activities of the investigated vegetal extractions.

There are very few and relatively recent studies concerning the identification of vaccine adjuvant qualities of certain vegetal extractions in animals. Thus, effects of some Chinese medicinal plants were compared to those of classical adjuvants such as Freund’s complete adjuvant (Wang D. et al., 2005). A mixture of vegetal extractions augmented both the in vitro blast transformation and anti-Newcastle disease antibody synthesis, being capable of acting on mechanisms involved in adaptive immunity.
The necessity of uncovering non-toxic adjuvants, with increased bioavailability and efficacy, as well as of possible extended use, represents a primary aim in modern veterinary vaccinology. Medicinal plants are, in this respect, an easily accessible source, with partially proved therapeutic effects.

The evaluation of adjuvant capacities of active principles from *Echinacea angustifolia*, a cultivated medicinal plant, renown (Percival S.S., 2000; Rehman J. et al., 1999; South E.H., Exon J.H., 2001) for its immune activity was done in comparison with that of a similar, alcoholic extraction of *Calendula officinalis*, a plant widespread in Romania, but less studied as an immune stimulant (Chemli R. et al., 1990; Della Logia R. et al., 1994). It was also considered useful to gather information on the potential differences between immunobiological effects of extractions from different *Echinacea* species, *E.angustifolia* and *E. purpurea* respectively (M. Spînu, PhD thesis, 2005). The extractions were tested under booster type administration, in connection with the antigen, to monitor the eventual modulating effects on the immunological memory (adaptive side of the immune response).

The immune system of birds offers an attractive model for studying adjuvant activities of conventional or modern compound (Lowenthal J.W. et al., 1999; Davison TF., 2003). The involvement of ontogenesis in eliciting an immune response is recognized in birds (Giurgea Rodica, 1982; Glick B., 1991; Sharma J.M., 1997), but there is no exact data on the age differences in what concerns the simultaneous administration of thymus dependent antigens and potentially immune stimulating/modulating (adjuvant) vegetal extractions in these species.

Various ways of antigen presentation to the cells of the immune system, depending on the administration route were already mentioned (Janeway, C.A. et al., 1999). Use of thymus dependent antigens to elicit adaptive immune responses was, in the given circumstances, accompanied by the administration of the extractions monitored for their adjuvant capabilities, on two routes – injectable and peroral, with the recording of immunological parameters. This approach allowed a final selection of the optimal route to increase the extractions’ adjuvant capacities.

Due to their multiple stimulating/modulating qualities, vegetal extractions represent a promising, worth to consider perspective, not only in vaccine industry but in therapy of veterinary preventive medicine as well.

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