SPECIFIC CELL-MEDIATED IMMUNE CHANGES INDUCED BY THE *IN VITRO* ADMINISTRATION OF VARIOUS VEGETAL EXTRACTIONS IN ANTIGEN PRIMED HENS

F. BRUDAŞCĂ, MARINA SPÎNU, CARMEN ŞANDRU

University of Agricultural Sciences, Faculty of Veterinary Medicine, Str. Mănăştur No. 3-5, Cluj-Napoca, Romania

Summary

A number of immunomodulatory effects have been attributed to the medicinal plants *Calendula officinalis* and *Echinacea angustifolia*; however, little is known about whether treatment with these plants can enhance antigen-specific immunity. Two groups of birds, a vaccinated group (n=25) and a saline injected control (n=35), were subjected to the blast transformation test. The birds were primed with two water-in-oil emulsified vaccines, against Newcastle disease, infectious bursitis, infectious bronchitis and viral arteritis (Quadractin) as well as against *Pasteurella multocida*, serotypes 1,3 and 5 (Cholerin triple), were administered subcutaneously, in a dose of 0,5 ml/bird, as antigen priming. The blast transformation test was done *in vitro,* using five alcoholic vegetal extractions (*Calendula officinalis, Echinaceea angustifolia*, *E. purpureea, Urtica dioica and Aloe vera*) and monitoring their effect in comparison with alcohol and/or no treatment, at each of the samplings, three weeks apart. The results indicated that, in the dosage applied *in vitro* and at the moments selected for the testing of blast transformation capacities, the alcoholic extractions of *Calendula, Echinaceea angustifolia*, *E. purpureea, Urtica dioica* and *Aloe vera* could not restore the level of cell mediated reactivity to its ante-priming values on short term. Still, antigen primed, vegetal extraction treated cells gave a better *in vitro* response. This effect was due to the combined treatment, not to the vaccination alone.

A number of immunomodulatory effects have been attributed to the medicinal plants *Calendula officinalis* and *Echinacea angustifolia*; however, little is known about whether treatment with these plants can enhance antigen-specific immunity. Investigations conducted on rats over a period of 6 weeks showed that the Echinacea treatment significantly augmented of the primary and secondary IgG response to the antigen. These results suggested that medicinal plants like *Echinacea* may enhance immune function by increasing antigen-specific immunoglobulin production (El-Gengaihi et all.,1998). In this context the stimulation of the oxidative burst as well as the modulation on monokine secretion were reviewed (Attele et all., 1999). Meanwhile, from the water or alcaline-water extracts of *Calendula officinalis L.,* polysaccharide fractions with molecular weights in the range of 25000 to 500000 and higher have been isolated, which, according to the granulocytes- and carbon clearance tests, showed significant immune stimulating activities. The isolated compounds belong to the group of water soluble, acidic branched-chain heteroglycans (Mashaly et all., 2000).

Still, the results concerning biological activities of various plant extractions could be contradictory. Their influences on the non-specific cellular immunity of the mouse after intra-peritoneal, intravenous or per oral application were investigated. Under various conditions no effects on the immune system could be found using the carbon clearance test (Larsson et all., 1993).

The research presented aimed to evaluate the *in vitro* specific cell-mediated immunity, after antigen priming in adult hens.

Materials and methods

The tests were carried out on hens kept on an intensive breeding, laying hen farm of 1300 individuals. Two groups of birds, a vaccinated group (n=25) and a saline injected control (n=35), were subjected to the blast transformation test. Blood was sampled on sterile heparine (50 UI/ml), by puncturing the wing vein. Two samplings were performed, on the day of the primary antigenic stimulation and three weeks later.

Two inactivated, water-in-oil emulsified vaccines, against Newcastle disease, infectious bursitis, infectious bronchitis and viral arteritis (Quadractin) as well as against *Pasteurella multocida*, serotypes 1,3 and 5 (Cholerin triple), were administered subcutaneously, in a dose of 0,5 ml/bird, as antigen priming.

The blast transformation test was performed *in vitro,* using five alcoholic vegetal extractions (*Calendula, Echinaceea angustifolia*, *E. purpureea, Urtica dioica* and *Aloe vera*) and monitoring their effect in comparison with alcohol and/or no treatment. Cell growth was quantified by means of the glucose consumption technique. Part of the blood sample (100 ml) was diluted with four times the amount of RPMI 1640. The mixture was distributed in five wells of a 96-sterile-wellplate (100 ml per well). Eight variants were tested once for each individual animal, namely (1) untreated control culture, (2) phytohaemagglutinin-M (PHA) (1 µl per well) treated culture, (3–8) alcohol, and alcoholic extractions of *Calendula, Echinaceea angustifolia*, *E. purpureea, Urtica dioica* and *Aloe vera* (1.5 µl per well) treated cultures. The quantities of both PHA and antigens were established when using the same technique during preliminary studies as being the most effective *in vitro* for the tested species. The cultures were incubated for 48 h at 37.5°C and 5% CO2. Glucose concentrations were measured in the initial medium and in all variants at the end of the incubation period, using a standard (100 mg dl–1) glucose solution, by means of an orto-toluidine colorimetric test. To do this, 12.5 µl of the cultural supernatant were transferred to 0.5 ml of orto-toluidine reagent, boiled for 8 min, cooled suddenly in cold water and read in a spectrophotometer at 610 nm wavelength (Unico 2100, United Products Instruments, Inc., Dayton, NJ, USA), using the reagent as a blank. The transformation index (TI) was calculated as follows: TI%=[(MG–SG)/MG]´100, where TI=blast transformation index, MG=glucose concentration in the initial culture medium and SG=glucose concentration in the sample after incubation.

Mean values, standard deviations and the statistical significance of the results (Student t test) were calculated.

Results and discussions

Resistance to diseases is a multigenic trait governed mainly by the immune system and its interactions with many physiologic and environmental factors. In the adaptive immunity, T cell and B cell responses, the specific recognition of antigens and interactions between antigen presenting cells, T cells and B cells are essential. It occurs through a network of mediator proteins such as the molecules of the major histocompatibility complex (MHC), T cell receptors, immunoglobulins and secreted proteins such as the cytokines and antibodies. The interaction of disease resistance with production traits and the environment is crucial therefore thorough studies aim to find ways to clarify its mechanisms and means of control (Masteller and Thompson, 1994). Vegetal extractions could be an easy-to-obtain, biologically available help in this direction.

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 (Dewick, 1997; Matthews et al., 1999).

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 artificialized breeding/exploitation of the domestic birds and animals, connected to an often disbalanced and incorrect nutrition (Zarnea, 1990; Mizoguchi et al., 1987; Muntean, 1990; Schulz et al., 1998). Nevertheless, numerous extractions of various plants are currently being used to augment the natural resistance to infections or in their treatment. Moreover, active principles of plants serve to partially restore the immune reactivity in indivisuals with innate immunosuppression (Bauer, 1996, 2002; Benecia et al., 1995; Bezanger-Beauquesne, 1993; Bezanger-Beauquesne et al., 1990; Bussing et al., 1997; Candinas 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, 2000; Kaufman 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.

The results of the blast transformation tests were presented in table 1.

Table 1

Mean values ± standard deviations (%) of the stimulation indices in the blast transformation test

	M	Al	PHA M	Cal	Ea	Ep	U	Aloe
sampling I	91.25± 6.25	88.48± 4.00	89.69± 3.56	89.82± 7.89	88.57± 10.09	89.82± 11.20	78.08± 3.54	85.98± 3.67
sampling II - Vacc.	82.71± 3.78	81.14± 5.45	74.67± 3.23	71.18± 5.34	72.31± 11.2	66.64± 2.22	68.90± 4.15	71.15± 1.91
sampling II - Control	74.75± 2.89	75.47± 3.45	64.87± 4.33	57.80± 3.90	50.22± 4.03	51.66± 5.77	45.53± 2.91	61.91± 6.30

Legend: Vacc- vaccinated group; variants: M- control, Al-alcohol, PHA M– phytohemagglutinin, Cal- *Calendula officinalis* extraction, Ea- *Echinacea angustifolia* extraction, Ep- *Echinacea purpurea* extraction, U- *Urtica dioica* extraction, Aloe- *Aloe vera* extraction

At the first sampling, the stimulation indices were very close, independently on the experimental *in vitro* variant. Still, the *Calendula officinalis* and *E.purpurea* extractions exerted the most pronounced effect, in comparison to the solvent control while the *Urtica dioica* extraction was the most inhibiting. Such results in chickens were previously reported by other researchers (Sandru et all., 2004, 2005; Spinu et all., 2004)

During the second *in vitro* testing, in both groups, the overall blast transformation indices decreased, more in the non-vaccinated than in the vaccinated group. The response to the *in vitro* treatments was much better in the antigen primed group, still, all the tested extractions exerted an inhibiting role, when compared to that of the alcohol. When looking at the stimulation indices obtained for the PHA M, a well-recognized stimulating compound, their low level might indicate the strong influence of injection (saline or vaccine) stress in the two groups. The best results were obtained with the alcoholic *Echinacea angustifolia, Calendula officinalis* and *Aloe vera* extractions. At equal doses, an active principle-dependent mechanism of action was obvious.

	M	Al	PHA M	Cal	Ea	Ep	U	Aloe
sampling I	91.25± 6.25	88.48± 4.00	89.69± 3.56	89.82± 7.89	88.57± 10.09	89.82± 11.20	78.08± 3.54	85.98± 3.67
sampling II - Vacc.	82.71± 3.78	81.14± 5.45	74.67± 3.23	71.18± 5.34	72.31± 11.2	66.64± 2.22	68.90± 4.15	71.15± 1.91
sampling II - Control	74.75± 2.89	75.47± 3.45	64.87± 4.33	57.80± 3.90	50.22± 4.03	51.66± 5.77	45.53± 2.91	61.91± 6.30

Conclusions

The results indicated that, in the dosage applied *in vitro* and at the moments selected for the testing of blast transformation capacities, the alcoholic extractions of *Calendula, Echinaceea angustifolia*, *E. purpureea, Urtica dioica* and *Aloe vera* could not restore the level of cell mediated reactivity to its ante-priming values on short term. Still, antigen primed, vegetal extraction treated cells gave a better *in vitro* response. This effect was due to the combined treatment, not to the vaccination alone.

References

1. **Şandru Carmen, Spinu Marina, Brudasca Gh. F., Duca Gh.** – 2004 – Influenta extractului alcoolic de urzica asupra capacitatii de transformare blastica leucocitara la puii broiler Analele Universitatii din Oradea, p. 231 - 237.
2. **Şandru Carmen Dana, Spînu Marina, Brudaşcă Gh.F.** – 2005 - Ierarhizarea funcției imunomodulatoare a unor extracte alcoolice vegetale în baza potențialului lor blastogenetic in culturi de leucocite de la păsări cu boala lui Marek Bul. Soc. Nat. de Biologie Celulara, nr. 33, p.157
3. **Spinu Marina, Şandru Carmen, Brudasca Gh. F., Giurgea Rodica, Tîrla Felicia**– 2004 – Blast transformation dynamics in chickens infected with bursitis virus and in vitro treated with hippophae rhamnoides L. Simpozion FMV Iasi, 10-11 iunie, Lucrari Stiintifice Vol. 47 (6) Medicina Veterinara, Iasi, ISSN 1454-7406, p. 595
4. **Attele AS, Wu JA, Yuan CS** - 1999 -Ginseng pharmacology: multiple constituents and multiple actions. Biochem. Pharmacol 58: 1685–1693
5. **El-Gengaihi SE, Shalaby AS, Agina EA, Hendawy SF** (1998) Alkylamides of *Echinacea sp.* purpurea L. as influenced by plant ontogony and fertilization. J Herb Spices Med Plant 5: 35–41
6. **Kaufman PB, Cseke LJ, Warber S, Duke JA, Brielmann HL** -1999-Natural Products from Plants. CRC Press, Boca Raton, FL
7. **Larsson A., Balow R.M., Lindhal T.L., Forsberg P.O.** – 1993 – Chicken antibodies: taking advantage of evolution, Poultry Sci., 72 (10):1807-12.
8. **Mashaly, M.M., Heetkamp, M.J., Parmentier, H.K., Schrama, J.W.** - 2000 - Influence of genetic selection for antibody production against sheep blood cells on energy metabolism in laying hens, Poult Sci., Apr; 79(4):519-24.
9. **Masteller E.L., Thompson C.B.** – 1994 – B cell development in chicken, Poultry Sci., 73(7):998-1011
10. **Bauer R.** -1996- Echinacea drugs effects and active ingredients Z Arztl Fortbild (Jena); 90(2):111-5.

11. **Benecia F., Courreges M. C., Nores M.M., Coulumbie F.C.-** 1995 – Immunomodulatory activities of Cedrela tobeflora leaf aqueous extract, J.Etnopharmacol., 49: 133 – 139.
12. **Bezange – Beauquesne, L.** et al. – 1990 - Les plantes medicinales des regions temperee, Malonie, Ed. Paris.
13. **Bezanger – Beauquesne, L.** – 1993 – Nouveautes en phytotherapie, Plantes medicinales at phytotherapie, 26 (3), 234 – 264.
14. **Bussing A., Suzart K., Schweizer K.** - 1997 - Differences in the apoptosis inducing properties of Viscum album L. extracts, Anticancer Drugs, 8 Suppl 1:S9-14.
15. **Candinas D., Mark W., Keaver V., Miyatake T., Koyamada N., Hechenleitner P., Hancock W.W.** - 1996 - Immunomodulatory effects of the alkaloid sinomenine in the high responder ACI-to-Lewis cardiac allograft model, Transplantation, 62:1855-60.
16. **Dewick P.M.** - 1997- Medicinal Natural Products. John Wiley & Sons, West Sussex, UK
17. **Matthews H.B., Lucier G.W., Fisher K.D.** -1999- Medicinal herbs in the United States: research needs. Environ Health Perspect 107: 773–778
18. **Muntean, S.** – 1990 – Plante medicinale şi aromatice cultivate în România, Ed. Dacia, Cluj-Napoca 22, 270-271.
19. **Mizoguchi Y., Katoh H., Kobayashi K., Yamamoto S., Morisawa S**. - 1987 - Effects of extract of cultured Lentinus edodes myceli (LEM) on polyclonal antibody response induced by PWM, Gastroenterol.-Jpn., 22(5):627-32.
20. **Schulz V., Ha¨nsel R., Tyler V.** -1998- Rational Phytotherapy: A Physician's Guide to Herbal Medicine. Springer- Verlag, Berlin
21. **Vogel F.R.** – 2000 – Improving vaccine performance with adjuvcants; Clinical Infectious Diseases; 30(suppl.3); 266-270.
22. **Zarnea G.** – 1990 – Tratat de microbiologie generală, vol.IV – Imunologie, Ed.Acad.Române, p.391-406; pag. 783-790