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Paramunization by pox virus inducers (non immunizing vaccines) as a new concept in prophylaxis and therapy in equine medicine

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Introduction

The terms „paramunity", „paramunization" and „paraspecific vaccines" are new and thus open to discussion. The terms embrace certain new prophylactic and therapeutic measures covered by a single principle. This is both appropriate and necessary, since there are a number of new diseases which can only be partly combated by the methods of classical medicine: infectious factorial diseases, mixed infections, chronic and recurrent diseases, therapy-resistent bacterial and viral infectious diseases, tumors, immune diseases and lowered resistance of an organism by immune-suppressive noxae or disregulation of the immune system with the known pathological consequences.

The equine immune system consists of an antigen-specific part and an antigen-nonspecific part (figure 1). The two parts are cross-linked and so form an uniform organic system. The antigen-specific mechanisms are responsible for building up **immunity**, while the antigen-nonspecific mechanisms are responsible for building up **paramunity**. Accordingly, for both historical and functional reasons the antigen-nonspecific part of the defence is known as the **paraspecific (innate, primitive) immune system**.

The development of a **non-immunising, regulatory, so-called "paraspecific" vaccine** is new but not surprising and rather a consequence of the fact that most of the body's immune defences, the so-called non-specific or paraspecific activities, have not yet been exploited. This development utilizes recent molecular-biological findings regarding the

structure and function of the complex immune system and makes this important potential of the body's own defence system available for prophylaxis and therapy. The insights gained with respect to paraspecific activities also made it impossible to continue to adopt the view the autonomy of the immune system hedl previously. Via its cellular and humoral mechanisms, the reactions of the immune system are closely bound up with those of the nervous, hormonal and vascular system. It is the virus-non-specific, so-called paraspecific, cellular and humoral elements, above all the **cytokines**, which, as regulatory molecular transmitter substances, link up the individual body systems. They include the **interferons** and **interleukins** in the immune system, the **selectins** in the vascular system, the **neurotransmitters** and **endorphins** in the nervous system (23, 24) or the **cortisols** in the hormone system (8, 16). Close synergetic and antagonistic interactions take place between all these **molecular transmitters** and these interactions can be **regulated** by the activation of the paraspecific part of the immune system (Fig. 2).

Activation of the paraspecific component of the immune system, i.e. **paramunisation**, leads to the development of **paramunity**. So-called **paramunity inducers**, or (according to a more recent definition, analagous to the designation of conventional, specific vaccines) **non-immunising, regulatory, paraspecific vaccines**, are used for this purpose (figure 3; table 1). Paramunisation is intended to bring about optimal functioning of the paraspecific cellular and humoral defence mechanisms (restoration). Depending on the patient's initial situation, it can be functionally **activating** (e.g. increased phagocyte formation), **regulatory** (e.g. induction of certain interleukins, interferons *inter alia*), or **reparatory** (e.g. suppression of IgE production, feedback mechanisms), i.e. it has a **restoring** effect overall, with the regulatory activity dominating. Usually, the result is a rapid increase in non-specific defence and improved interactions in the network of the non-specific and specific immune system. Accordingly, **paramunisation** or vaccination with non-immunising but regulatory vaccines is understood as the medicinal activation and regulation of cellular and humoral elements of the paraspecific component of the immune system. This is connected with the production or release of cytokines with the aim of eliminating dysfunctions and repairing deficits. This means that, depending on the kind of

paramunisation and reaction situation or patient's general condition, certain of the body's own paraspecific defence mechanisms may be intensified, complemented or even suppressed.

Paramunity inducers from attenuated and inactivated poxvirus satisfy the demands made on these new medicines with respect to their effects and harmlessness to an especially high degree.

With the help of monoclonal antibodies, it can be shown that pox viruses have structural regions on their surfaces (epitopes or protein regions) which are responsible for what is known as paraspecific immunisation (paramunisation), not for specific immunisation. Through attenuation via several hundred cell-culture passages and additional inactivation, the pox viruses lose their specific immunising properties, whilst the paraspecific properties even increase (competitive phenomenon). The structural clarification of the coat proteins of the pox viruses makes it possible to isolate the paraspecific proteins, to visualise them biogenetically and functionally capture their effects. They activate macrophages, NK cells, native T lymphocytes and other lymphoreticular cells systemically and locally, as a result of which a large number of cytokines (cytocascade) are simultaneously produced and released, *inter alia* interferon α and γ, IL-1, IL-2 and IL-12, CSF, TNF.

1. Production of paraspecific vaccines (paramunity inducers) from pox viruses

Highly attenuated animal pox strains, harmless for humans and animals, were used exclusively to produce the paramunity inducers. Attenuation took place via several hundred continuous passages in permissive cell cultures in accordance with the plaque-dilution method (11). The required period of time was 15 to 20 years. During the continuous cell-culture passages, stable virus clones develop via mutations and selections. They differ **biologically** from the heterogeneous source strains through the loss of virulence, **physically** through the decrease in molecular weight and **chemically** through a large number of deletions. The latter concern structural components

responsible for virulence, host spectrum and specifically immunising properties. The phylogenetically old, paraspecific structural units, in contrast, are preserved.

The following attenuated animal pox strains were used:

- **parapoxvirus ovis**, D 1701 strain (135 passages in embryonal lamb-kidney cultures, 137 passages in embryonal bovine lung , 49 passages in MA-104 cells and 62 passages in vero cells),
- **avipox, fowlpoxvirus**, HP-1 strain (447 passages in chicken embryo fibroblast cultures (FHE),
- **canary poxvirus,** KP-1 strain (567 passages in (FHE),
- **orthopox, vaccinia virus**, MVA strain (572 passages in FHE).

In addition, the attenuated pox strains were inactivated (0.05% β-propiolacton) in order to ensure that any small amounts of specifically immunising activities that might still be present were rendered inactive. In pox research, it is a long-established fact that inactivation of pox viruses destroys their immunising effect. Through this combination of attenuation and inactivation, biopreparations are obtained that can be applied as often as required without the risk of side effects or the induction of neutralising antibodies.

The production of paramunity inducers took place in a similar manner to that of conventional vaccines (13). Cell-culture virus harvests with a minimum virus titre of $10^{7.5}$ KID_{50}/ml. We used high-speed centrifugation or fractionated ultracentrifugation via sucrose gradients for batch cleansing purposes. The cleansed and inactivated virus material was mixed with 2.5% polygeline and filled into 1.0 ml bottles, lyophilised and stored at $+4^0$C. The lyophilisate is always dissolved in sterile aqua dest. prior to use. The preparation contains at least 640 EU/ml (effective units in the baby mouse challenge test with stomatitis vesicularis virus) (14, 15).

The inducers were produced as **monovaccines** from one virus strain each (working name „PIND-AVI“ and „PIND-ORF“) or as **combination vaccines** from several attenuated viral strains of varying genera (working name „Conpind“).

Duphapind® (PIND-AVI) and **Baypamun®** (PIND-ORF) have been available to equine medicine as approved paraspecific monovaccines for years. Since then, they have been regularly used on horses successfully and without any side-effects. They can be used together with antiobiotic therapy or chemotherapy and, temporarily, even with cortisone preparations without any problems.

The pox inducers are non-toxic and pyrogen-free. Parenteral (i.a. subcutaneous) application to induce a systemic effect and local (cutaneous, oral, intranasal, rectal, intravaginal) application via the skin and mucous membranes are both possible. Local application activates the local, paraspecific defence mechanisms above all. The pox inducers are immediatelyx metabolised and leave no residues behind. Minimal antibodies demonstrable via ELISA occasionally produced after repeated and long-term use do not neutralise the efficacy of the preparation.

3. Results of multicentric, randomised studies on the efficacy and harmlessness of paraspecific vaccines from attenuated pox viruses (pox inducers) in horses

The paraspecific, non-immunising vaccines described above (e.g. Duphapind® and Baypamun®) have been commercially available for veterinary use as so-called **paramunity inducers** for years. With respect to their efficacy and harmlessness, they have meanwhile proved extremely valuable in the prevention and therapy of equine virus infections, chronic infections and for rapid optimisation of the defence system, in particular in newborns and patients with reduced immunity.

As with the specific vaccines, body weight is irrelevant for the use of paraspecific vaccines. The only requirement is that an inducer dose, as with conventional vaccines, contains a minimum amount of effective units (> 160 EU/ml), i.e. the inducer quantity administered must be sufficient to achieve the threshold value for activation of the paraspecific component of the immune system in the vaccine. Through application of the inducer, a chain reaction, "acceptor cell – cytokine elution – effector cell" – is set in motion, which (e.g. in the case of parenteral application) affects the entire organism.

The necessary threshold value has been demonstrated by means of a dose-efficacy ratio (dose-efficacy curve) in animal tests and standardised.

For systemic application (normal method of administration), application of the paramunity inducers was subcutaneous or intramuscular. In cases where the disorder was solely local or restricted to the mucous membranes, administration of the pox inducers was also intranasal, cutaneous, oral, intravaginal or rectal in the form of a non-dissolved pellet (immediately absorbed).

Data concerning more than 10.000 horses paramunised regularly (some over the entire period) during the past 10 years were evaluated in terms of **harmlessness** and **efficacy**. The group included both so-called healthy animals using the inducer as a prophylaxis and horses with a specific disease. No local or systemic postvaccinal impairment or complications were observed in either of the two groups after one-off or repeated application -over several days or at regular intervals- of inducers approved for use in the veterinary field such as Baypamun® or Duphapind® or laboratory preparations such as Conpind AOV or MOV. Simultaneous therapy with antibiotics, chemotherapeutic agents, cytostatic drugs or cortisone preparations did not lead to any side-effects but enhanced their effects instead (empirical evidence).

The clinical findings are compiled in Tab. 2. **Prophylactic application** proved its worth in all cases in which there was risk of immunosuppression, dysregulation or a temporarily compromised defence system as the result of various situations such as transport, specific pressures, infections, operations etc.

A very important prophylactic indication concerns the prevention of the immunosuppressive effect of transportation (16, 25). The effect of Baypamun® in preventing or reducing the increase of serum cortisol of transport induced stress was examined using 10 horses in four similiar transport tests. Untreated horses were subjected to the same transport as controls. Serum concentrations of cortisol were measured before loading and after transport. Baypamun® was administered intramuscularly on the second and fourth day before journey. The mean resting serum

concentration for the controls was 1.78 µg/100 ml, shortly before loading this rose by 61% and during transport the concentration increased by further 8.5%. The average value in the pretreated resting horses was 1.42 µg/ml. There was no significant increase before loading and during transport indicating that transport stress can be prevented by Baypamun®.

The **therapeutic efficacy** of the paramunity inducers is especially impressive and also unexpected. In general, the pox inducers proved their worth in the treatment of viral infections and chronic forms of infection of varying genesis. The numerous, empirically obtained data are statistically meaningful in particular for all virus infections (chronic and also acute forms) and even infectious factor diseases, mixed infections such as infectious diseases of the respiratory tract and neonatal infections.

Endotoxaemias may also constitute a further important field of indications. Currently, however, the only results available are those from experiments involving mice.

The findings after local therapeutic use of pox inducers are even more impressive: wounds healed more rapidly, extensive stomatitis disappeared within days, circumscribed erosions (vesicles) in the mouth even within a few hours, papillomas receded, even inflammatory processes in the urogenital area were successfully treated without any additional specific therapy. Overall, therapeutic efficacy could be documented with respect to all the pathological processes whose primary cause was a dysfunction of certain activities of the defence system or the entire defence potential.

With respect to the immunological parameters, a uniform picture can be ascertained in almost all patients. In a majority of the paramunised patients, the immunological parameters were reduced; after treatment, they returned to the normal range. Paramunisation reduced elevated values to normal levels. In cases involving normal values, no changes took place.

The following conclusions can be drawn from the **data obtained within the framework of multicentric, randomised studies using the same inducer batch:**

treatment with paramunity inducers from pox viruses activates the paraspecific defence system within only a few hours and has a general, regulatory effect on the immunological parameters in the direction of homoeodynamics. Paramunisation must therefore be seen as a restorative process or biological regulation designed to optimise immunological parameters. The clinical effects are correspondingly positive. What is important in this connection is the finding that „normal" parameters remain unchanged. There is therefore no risk of overreactions.

4. Summary

The so-called primitive, innate or paraspecific defence system is the phylogenetically older part of the complex immune system. It enables the organisms to immediately attack various foreign substances, infectious pathogens, toxins and transformed cells of the organism itself. „Paramunity" is defined as an optimal regulated and activated, antigen-nonspecific defence, acquired through continuous active and succesful confrontation with exogenous and exogenous noxes or by means of „paramunisation" with so called „paraspecific vaccines" („paramunity inducers"). Paraspecific vaccines based on different pox virus species (e.g. Baypamun ®, Duphapind®, Conpind) have turned out to be effective and safe when applied with horses. Pox virus inducers activate phagocytosis and NK-cells in addition to regulation of various cytokines, notably interferon α and γ, IL 1, 2 , CSF and TNF which comprise the network of the complex paraspecific immune system.

The results of as well experimental work as practical use in equine medicine have shown that paramunisation by pox inducers goes far beyond the common understanding of so-called „immuno-therapy". They are „bioregulators", because they have 1. a regulatory effect on a disturbed immune system in sense of a physiological normalisation, and 2. simultaneously an regulatory effect between the immun, nervous, circulatory and hormon system. Therefore, the use of paramunisation by pox inducers opens a new way of prophylaxis and therapy, not only with regard to infections, but also with regard to different other indications.

5. Literature

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Table 2:

Use of poxvirus inducers in veterinary medicine
- indications in horses -

Indication	Reference
infectious diseases of the respiratory tract	Strube et al. 1989 Ziebell et al. 1998
herpes virus 1 infections rhinopneumonitis (neurological course)	Dambacher 1992/1993 Hechler 1981 Thein et al. 1981
cortisol increase of foals at weaning	Lindner et al. 1993
neonatal infections	Rademacher 1992 Enbergs et al. 1994 Mayr 1998
transport induced increase of serum cortisol	Mayr & Siebert 1990 Siebert 1988
infectious skin diseases	Mayr 1998
chronic infectious diseases of different aetiology	Mayr 1998
poxvirus infections	empirical data
tumours of different aetiology	empirical data
support of vaccinations	Mayr 1998

Figure 1: **Simplified diagram of the structure of the immune system**

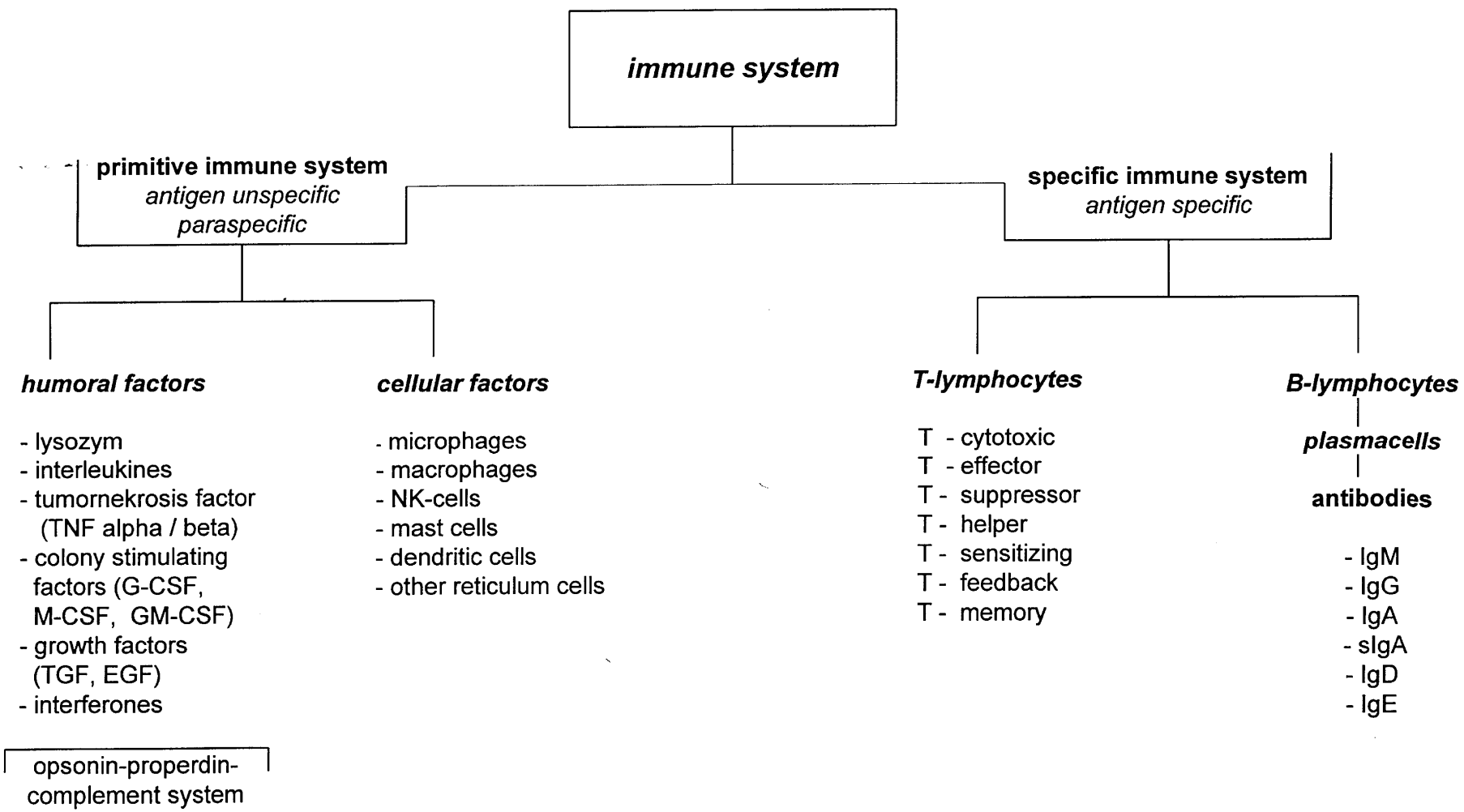

Figure 2: **Interactions of the immune system with other essential systems of the organism**

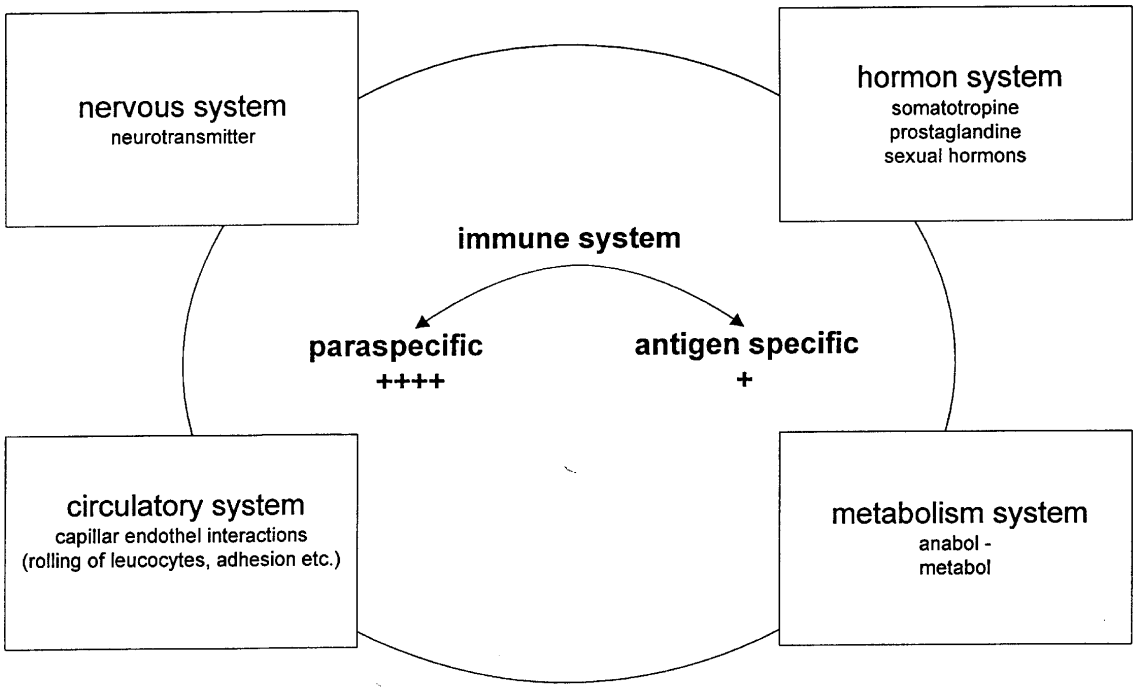

Figure 3: **Exploitation of the immune system in medicine**