ACUTE PHASE REACTANTS, CHALLENGE IN THE NEAR FUTURE

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Introduction

By many different scientists from various disciplines the acute phase reaction known to occur on infection, inflammation, trauma, burns, malignancies and tissue damage in general, has been studied. Last decade emphasis has been given to application of tests for acute phase reactants to monitor animal health in general, as well as for human patients suffering from specified classes of diseases. However, basic mechanistic patterns associated with biological reaction patterns still remain to be discovered, such as local production of acute phase proteins by cells of organs involved in specific physiological mechanisms and disease processes. This may be associated with defence functions and with development of, even worse, tissue alteration.

Precolostrum mammary tissue (McDonald *et al*, 2001) and mastitic mammary epithelium have been shown to form mammary serum amyloid A (mSAA) (Vivanco *et al*, 2002; Nguyen *et al*, 2002, Balciute *et al*, 2005). Moreover, haptoglobin (Hp) and other acute phase reactants are found in the milk. These factors are supposed to have functions in regulating the inflammatory process and to be beneficial for the enteric milieu of the young mammal including protection of the gut mucosa by mucus formation. In birds with chronic arthritis the synovial cells may reveal SAA upregulation, SAA protein formation (Ovelgönne *et al.,* 2001; Upragarin *et al*, 2002, 2005a) and amyloid formation (Landman, 1998, Upragarin *et al*, 2005a).

The present paper facing future developments expected, will be based on i. present scientific activities concerning basic mechanistic patterns not discovered sofar, ii. development of technology to measure quantities of proteins or cellular upregulation of their formation, and iii. assessment of disorders, health and welfare, and iv. it will regard some topics in these fields.

Basic mechanistic patterns

Specific organs

Differentiated reactivity patterns of the parenchymal cells per organ involved, such as mammary gland, will depend on cytokines locally active and are to be unraveled. Moreover, cell specific factors may be liberated. Just like enzymes known from clinical enzymology, specific cell proteins such as the fatty acid-binding gut protein (Niewold *et al*, 2004), may be discovered and find their applications in diagnostics and therapy.

Functional aspects of acute phase reactants in milk and blood need further attention. When activities which activate or just mitigate the inflammatory reaction, are known, this knowledge can lead to new ways for therapy and prevention of inflammatory processes in organs involved, such as lung or mammary gland.

Local SAA formation and its precipitation as amyloid as has been found in avian amyloid arthropathy (Landman, 1998), need further investigations. From the pathogenetic mechanisms preventive measures may be developed. Once the beta-pleated sheets of amyloid have been formed, the substance has tremendous food safety implications. In murine studies orally administered AA-amyloid appeared to enhance inflammation- / acute phase reaction-induced amyloidosis, whereby the administered material acted as nidus for amyloidogenesis. This indicates a strong ban of this pathological material for risk groups of consumers comparable to that of prions. In mammary tissue, colostrum and fresh milk corpora amylacea may occur which contain amyloid (described to be derived from casein [Niewold *et al*, 1999]). Recently this amyloid was found to be positive for SAA (Toussaint *et al*, 2004; Balciute *et al*, 2005), and thus, as well as locally formed mSAA as unwanted beta pleated factors in colostrum and milk related to this SAA, need to be investigated.

Various proteins

In birds acute phase proteins such as (ovo)transferrin appear to have special characteristics differing from mammals. In mammals and possibly in avian species as well, some acute phase proteins may reveal differences in glycosylation patterns associated with different diseases and stages of those diseases, as has been shown for feline α1-acid glycoprotein (AGP). Further analysis may unravel basic biological mechanisms, indicate specificity of the glycosylation patterns for disease and reveal new concepts for therapy.

Reaction of different analytes in various situations, separate and combined

Viral, bacterial, and protozoal infections may be associated with different patterns in cytokine release and acute phase reactivity. Inflammatory processes in internal organs appeared to result in more severe reactivity patterns than diseases of the skin and the enteric system. Specific knowledge of pattern details may lead to implication of the parameters in diagnostics and stageing of the disease.

Calculation of an index from values of rapid- and slow-reacting positive and negative APPs has been repeatedly mentioned (Gruys and Toussaint, 2001; Gruys, 2002; Tousaint *et al.,* 1995, 2002, 2004; Niewold *et al*., 2003), because it appeared to increase statistical sensitivity and specificity for detecting non-healthy subjects. It covers a broad time span and includes changes in blood values resulting from the body's reactivity as well as starvation. In layer chickens on induction with *Staphylococcus aureus* or turpentine an acute phase protein reaction was induced. Measurement of values for SAA, transferrin, serum albumin and apolipoprotein A-1 in blood samples of these birds (Upragarin *et al*., 2005b) showed that calculation of an acute phase index, offers promising results in this species. Outcome was as has been calculated for cows with various diseases (Toussaint *et al.,* 1995) and for pigs with a *Streptococcus suis* infection (Toussaint *et al.*, 2002).

Infections and vaccination

To prevent spontaneous disease often vaccination is propagated. It has been shown, however, that upon vaccination an acute phase protein reaction may develop. This appears to limit the profitability of vaccines, because acute phase reactions are known to be contraproductive in view of muscle anabolism. Future interest will be more on amino acid pattern differences in muscle and APPs (Table 1) and on the negative acute phase reactants.

On starvation and negative energy balance associated with most diseases, muscle proteins are catabolised for amino acid supply of the hepatic APP formation and as source of energy. Especially for those APPs which rapidly and quantitatively increase in blood, their formation may have amino acid impact. An increased hindquarter protein catabolism exceeding the hepatic protein synthesis, and efflux of glutamine and alanine from the hindquarter was measured during a porcine induced endotoxemia study (Bruins *et al.,* 2003). For growth during and after recovery from a disease, food requirements for amino acids thus may differ from the formula in ordinary food. Some pig studies indicate positive influences of additional dietary tryptophan (Le Floc'h *et al.*, 2004) or L-arginine (Bruins et *al.*, 2002).

Negative APPs may be associated with a change in concentration of bound compounds. A decrease of retinol-binding protein and of vitamin A values may be *vice versa* interrelated, vitamin A-deficiency being well known to decrease immune reactivity of children in developmental countries. It is striking to encounter a huge negative variation from normal blood vitamin A values of around 1-0.75 μMol/l to around <0.1 μMol/l in fattening pigs, as was revealed in a local investigation by a Dutch practitioner (Hogendoorn, 2004).

In cattle an association of APPs with parturition, starvation and ketosis has been described. Rise in non-esterified fatty acids (NEFAs) occurs; their level increase might parallel those of some APPs. It is to be expected that due to negative APPs, blood vitamin A levels decrease as suggested for the pig. The NEFAs are toxic and have a negative influence on metabolism. It is hypothesized that the NEFAs may decrease on increased muscular acitivity (walking). An inversive association with walking activity has been shown (Adewuyi, 2004).

Table 1. Amino acid composition differences between muscle protein and major acute phase proteins after a gross amino acid composition list (Reeds *et al*, 1994)

Amino acid	*skeletal muscle protein*	*CRP*	*SAA*
Phe	40	105	103
Tyr	36	50	67
Trp	13	42	45
Arg	69	36	116
Ala	59	31	106

Development of technology to measure quantities of proteins or cellular upregulation of their formation
After radio-immunoassays (RIA) and enzyme-linked methodology (ELISA) at present several groups develop methods for rapid measurements of APPs. Nephelometry, turbidimetry, and other methods on liquid phase analysis, and protein array methodology on slides, as well as 3D electrophoresis with MAS spectrometry have been shown to be applicable on samples with acute phase reactants. Especially these technological developments are of crucial importance for the future. When rapidly and with low costs many samples can be handled, the APPs have a diagnostic future.

Assessment of disorders, health and welfare
Specific groups of patients, such as castrated horses, cows with mastitis, periparturient sows, sheep with mastitis, or dogs and cats with infectious disorders have benefit form acute phase reactant measurement. When larger groups of animals are involved and this may concern a more wide variety of diseases, multi-analysis technology coupled with pattern recognition software has the power of selective diagnostics.
At least, analysis of reaction pattern differences on the same agent may be used for selection and breeding purposes.

Conclusion
Future possibilities for acute phase reactants depend on basic new mechanistic findings of known proteins, new discoveries such as organ specific components, and on technological possibilities for rapid chemical multianalyses with computer analysis of the patterns found. The shared cost EU project (number QLK5-2001-02219) on porcine acute phase proteins has helped to spread the knowledge to specified scientists of member states involved, and to develop a base for practical applications. For other species such as cattle, horse, dog and cat, but also chicken and even human, a similar field is open for development of clinical and health management applications.
Finally, new fields of research and application are in the negative metabolic influences of acute phase processes and their relationship with growth and nutrition.

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