Proc. Soc. Nutr. Physiol. (2011) 20

Energy demand and energy metabolism in the immune system (Energiebedarf und Energiemetabolismus im Immunsystem); B. Kaspers - München

In order to perform its main function, the control of infections and tumours, the immune system has developed as an extended network of interacting soluble and cellular components. During the last 50 years significant progress has been made in the identification and functional characterization of the immune cells, soluble signals and effector molecules involved. From this work it became apparent that the immune system is not only controlled by genetic factors but also by a broad range of environmental determinants. Among them, nutrients play a particularly important role (Fernandes 2008) which is best shown in cases of malnutrition. Both over- and undernutrition greatly impact on the function of the immune system (Scrimshaw and SanGiovanni 1997). Beside the lack of single amino acids, vitamins, minerals and micronutrients (Cunningham-Rundles and others 2005) protein energy malnutrition (PEM) has been known as a major reason for increased susceptibility to infection in man for a long time (Schaible and Kaufmann 2007). However, the mechanisms underlying this link could not be further analysed until the discovery of the B- and T-cell system and its pivotal role in the defence of infectious diseases. Robert Good and co-workers were the first to study the impact of caloric and protein malnutrition on the function of lymphocytes and phagocytes in defined rodent models and found that dietary changes had different effects on the individual components of the immune system (Good and others 1976). In parallel with the progress made in immunology the interest in the role of nutrition on immune function increased and a range of nutrients with activating and inhibiting effects were identified (Klasing 2006).

In general, researchers have used two approaches to investigate how nutrients influence immune function. A large body of information was obtained in cell culture experiments where defined nutrients were either added to or depleted from the cell culture media. The impact of these nutritional conditions on the immune cells was analysed through quantification of cell specific activities such as phagocytosis of bacteria by macrophages and granulocytes, production of reactive oxygen or nitrogen intermediates or proliferation of lymphocytes in response to mitogenic or antigen-specific stimulation. Alternatively, experimental animals were fed defined diets and immune cell responses were analysed after cell isolation using the same functional parameters as described before. Through these studies a wealth of information was obtained on the role of amino acids (Li and others 2007) lipids (Yaqoob and Calder 2007) glucose (Fox and others 2005) as well as vitamins and trace elements (Maggini and others 2007) in the immune system.

The majority of studies discussed have only addressed the dietary modulation of selected functional parameters. To obtain a more comprehensive knowledge of the benefits and disadvantages of dietary components in immune function, infection experiments with a variety of pathogens have to be performed. However, such studies require biosecurity facilities and are therefore expensive to perform, thus greatly limiting the number of animals investigated in each single study. As a consequence *in vitro* and *ex vivo* studies are still standard. Importantly, with the increasing knowledge in immunology cellular parameters can now be investigated which provide a better correlation with immune cell development and function *in vivo.*

Protein energy malnutrition (PEM) has severe effects on the immune system. In children it was shown to cause atrophy of the thymus and consequently underdeveloped peripheral lymphoid organs (Savino 2002). It may further lead to reduced antibody responses with increased susceptibility to a range of bacterial infections (Woodward 1998).

Department of Veterinary Sciences, Institute of Animal Physiology, Faculty for Veterinary Medicine, Ludwig-Maximilians-Universität München, Veterinärstr. 13, 80539 München

Experimental models have further shown that the phagocytic system is functionally impaired with a diminished capacity to engulf and eliminate pathogens and a reduction in the antigen presenting capability of dendritic cells (Abe and others 2003). While PEM is of particular concern in developing countries overnutrition in industrial countries on the other hand poses a newly emerging public health threat which is still only partially understood (Wolowczuk and others 2008).

In domestic animals the dependence of the immune system on an adequate energy supply is best studied in ruminants during the periparturient period which is characterized by a sudden increase in energy requirements associated with an increase in the number and severity of metabolic and infectious diseases (Goff 2006). The latter is accompanied by changes in several immunological parameters affecting both the innate and adaptive immune system (Sordillo and others 2009). This is well documented in sheep where the so called periparturient relaxation of immunity is associated with an increase in gastrointestinal nematodes and faecal egg counts. Since energy demand increases rapidly during this period it was assumed that increased worm burden is a consequence of immunosuppression related to the negative energy balance. In this model homeostasis of energy substrates is altered in such a way that the immune system in undersupplied and thus functionally impaired (Goff 2006). This hypothesis has been addressed in the sheep model where faecal egg counts were compared in twin-rearing eves with an optimal and reduced protein supply. Dietary protein reduction led to a significant increase in worm egg shedding which could be reversed if one lamb was weaned on day 10 (Houdijk 2008). However, subsequent attempts to identify the immunological parameters responsible for the periparturient relaxation of immunity have provided conflicting results. No single parameter was identified up to date even though some studies point to a role of reduced mast cell and eosinophil numbers as well as immunoglobulin titers in immunocompromised eves (Beasley and others 2010).
Likewise, periparturient immunosuppression has been associated with a reduced health status in cows and a range of immunological parameters both on mucosal surfaces and in lymphoid organs and blood have been investigated. Mehrzad and colleagues observed that the number of neutrophils is significantly reduced in blood of periparturient cows and that adhesion and migration of these cells into the mammary gland is diminished. Curiously, the phagocytic activity of these cells was increased pointing to an improved functional activity. More detailed functional analysis however revealed that the respiratory burst activity was reduced leading to a decrease in bacterial killing (Mehrzad and others 2002). This work nicely underscores the necessity to evaluate a broad range of functional parameters in order to gain a comprehensive picture of the immune status at a given time point.
In addition to the function of these and other innate immunity cells, properties of lymphocytes were investigated by many groups in cows during periparturient period. Again, a significant reduction of T-cell frequencies in the blood was found and functional studies demonstrated a shift from an IFN-γ and IL-12 producing phenotype (as a readout of a so called T-helper 1 (Th1) response) to the production of typical Th2 cytokines including IL-4, IL-10 and TGF-ß (Kehrli and Goff 1989). IL-10 and TGF-ß are particularly interesting in this context since both are typical immunoregulatory cytokines down-regulating inflammatory responses induced by pathogen infection. Subsequent work has demonstrated that some of these immunosuppressive effects of a negative energy balance can be reverted by feeding additional energy substrates (Ohtsuka and others 2006; Stabel and others 2003).

Collectively these and many other studies (for review see (Carroll and Forsberg 2007; Schaible and Kaufmann 2007) have shown that an adequate energy metabolism is of particular importance for a fully functional immune system. Nevertheless, investigations of the underlying molecular mechanism have only begun recently. Immunologists have largely focused their efforts on the T-lymphocytes since these cells are master regulators of the immune response, essential in the development of immunological memory and transit rapidly between different functional phenotypes.

T-cells develop in the thymus (hence their name) where they undergo a selection process which leads to the generation of self tolerant cells while autoreactive T-cells are eliminated by apoptotic death. Thymic development is associated with vigorous proliferation of immature cells and thus critically dependent on energy substrates. PEM has been shown to cause thymic atrophy in children (Savino 2002). Mature T-cells leave the thymus and migrate to the secondary lymphoid tissues and mucosal surfaces. These naïve T-cells constantly circulate through the body leaving the blood stream to migrate into lymphatic tissues and re-entering the circulation. The migration and active maintenance

of cellular activity requires metabolic substrates. Deprivation of energy leads to apoptotic death which is an important mechanism in the homeostatic regulation of the T-cell pool (see below).
Upon encounter of their specific antigen, T-cells switch from a resting to a highly activated phenotype which is characterized by a significant increase in cell size and cell numbers. In addition, these cells start to perform effector functions such as the secretion of cytokines and the attack of infected cells and tumour cells. Activation of T-cells was shown to be associated with consumption of large amounts of energy substrates and a switch in the metabolic pathways utilized to generate ATP. Moreover, the rapid cell division (doubling time was calculated to be in the range of 6-8 hours) requires synthesis of new membranes and proteins and is therefore critically dependent on the supply of fatty acids and amino acids either from the environment or from *de novo* synthesis. As a consequence, activated T-cells are most sensitive to nutrient deprivation which ultimately leads to an immunosuppressive phenotype.
Finally, an antigen specific immune response is terminated as the infection is controlled and cells return to a quiescent state. During this process more than 90% of the activated T-cells die, leaving a pool of actively maintained memory T-cells. Again, this functional status still requires energy and must therefore be regarded as an active process (for more details see (Michalek and Rathmell 2010).

The processes described above can now be reproduced to a large extend *in vitro* by providing the appropriate molecular cues. The cues required to maintain naïve T-cells, to shift them to an activated stage by antigen encounter and to maintain memory cells have been and still are the topic of intense immunological research over the last five decades. The knowledge gained is now applied to investigate the role of metabolism in T-cell biology and is slowly extended to other cells of the immune system in particular to antibody producing B-cells.

Naïve T-cells consume glucose and other essential nutrients such as glutamine and fatty acids at a low rate to maintain housekeeping functions (Jones and Thompson 2007). In particular, glucose is utilized to generate ATP by oxidative phosphorylation as well as to produce lactate and for the synthesis of oligosaccharides. To maintain this metabolic phenotype naïve T-cells require extrinsic signals. Activation of the T-cell receptor (TCR) plays a key role in the expression of the glucose transporter Glut 1 and in subsequent glucose uptake. In the absence of TCR signalling Glut 1 is downregulated and thus the T-cell becomes deprived from glucose which ultimately leads to nutrient stress and apoptosis. It has been suggested that TCR signalling induces transcriptional upregulation of Glut 1 through stimulation of the mitogen-activated protein kinase (MAPK) and AMP-activated protein kinase (AMPK) pathways (Tamas and others 2006). In addition to the TCR, interleukin-7 (IL-7) serves as a critical homeostatic factor. It has long been know that in the absence of IL-7 signals T-cells fail to develop in the thymus, that naïve T-cells are not maintained in the periphery and that T-cell memory is impaired (Michalek and Rathmell 2010). One functional property of IL-7 is to promote Glut 1 trafficking to the cell surface thereby increasing glucose uptake. In the absence of IL-7 naïve T-cells are unable to maintain glycolysis *in vivo* and undergo apoptosis (Jacobs and others 2010). From this work it was concluded that IL-7 regulates T-cell homeostasis through the control of energy metabolism with TCR activation leading to Glut 1 synthesis and cytoplasmic storage and IL-7 inducing the trafficking of Glut 1 to the cell membrane.

In response to pathogens T-cells rapidly become activated and shift from the quiescent phenotype described before to a highly active state. To do so they drastically alter their metabolism in order to support the bioenergetically demanding processes of growth, cell division and effector function. The most dramatic change is the sharp rise in glycolysis which leads to a significant increase in lactate production a pathway termed aerobic glycolysis. It is assumed that T-cells can supply their energy needs rapidly in this way while providing important metabolites such as pentose sugars for nucleic acid synthesis and NADPH for reducing power and lipid synthesis at the same time. In addition, fatty acids and nucleic acids are no longer utilized for energy metabolism and thereby available for protein synthesis and membrane formation. It has been shown repeatedly that despite the presence of alternative metabolic fuels, glucose deprivation leads to reduced T-cell proliferation, deficient cytokine production and ultimately to programmed cell death (Cham and Gajewski 2005; Greiner and others 1994).
As discussed for the naïve T-cell external signals are required for T-cell activation. These include activation of the TCR, ligation of the costimulatory receptor CD28 and cytokine signals such as IL-

2R activation. TCR stimulation alone does not increase glucose metabolism to the extent required and therefore does not provide the energy resources for full activation. It should be noted, that TCR signalling without additional signals leads to anergy or apoptosis of the T-cell. It is the costimulation through CD28 which is required for maximal glucose uptake and glycolysis (Jacobs and others 2008) and rescue from apoptotic death. Again, Glut 1 has emerged as the most critical glucose transporter in this system which is strongly upregulated upon TCR/CD28 mediated activation. Growth factors such as IL-2 are critical to maintain the cell surface levels of Glut 1 during the ongoing immune response.

Once the invading pathogen is cleared, the antigen specific T-cell pool contracts and a small number of long living memory cells is maintained. The loss of TCR stimulation and growth factor signalling leads to a sharp decline in glucose metabolism. Part of this process is the loss of Glut 1 expression through internalization and degradation thereby depriving T-cells from external glucose supply (Wieman and others 2009). As a consequence, cells start to utilize alternative fuel sources such as intracellular components and membranes which are mobilized in a process called autophagy. However, this pathway is a self-limiting survivle strategy and the majority of cells undergo programmed cell death.

It is currently unknown how some cells avoid this fate to become long living memory cells. However, it is suggested that they selectively upregulate the IL-7 receptor and can thereby receive the signal required for nutrient uptake and metabolism (Kaech and others 2002).

In summary, for T-cell homeostasis and adaptive immunity, it is critical that T-cell metabolism adequately meets the demands of the specific T-cell functions. If it fails, autophagy, cell cycle arrest and apoptosis may ensue. On the other hand, excess metabolism may promote T-cell hyper-reactivity and autoimmunity. A greater understanding of how the metabolic pathways are regulated and function in lymphocytes may provide clues how to target metabolism for therapeutic approaches. It may also help to better understand immunosuppression in domestic animals during the status of negative energy balance.

References

ABE, M., AKBAR, F., MATSUURA, B., HORIIKE, N. & ONJI, M. (2003) Defective antigen-presenting capacity of murine dendritic cells during starvation. Nutrition 19, 265-269

BEASLEY, A. M., KAHN, L. P. & WINDON, R. G. (2010) The periparturient relaxation of immunity in Merino ewes infected with Trichostrongylus colubriformis: parasitological and immunological responses. Vet Parasitol 168, 60-70

CARROLL, J. A. & FORSBERG, N. E. (2007) Influence of stress and nutrition on cattle immunity. Vet Clin North Am Food Anim Pract 23, 105-149

CHAM, C. M. & GAJEWSKI, T. F. (2005) Glucose availability regulates IFN-gamma production and p70S6 kinase activation in CD8+ effector T cells. J Immunol 174, 4670-4677

CUNNINGHAM-RUNDLES, S., MCNEELEY, D. F. & MOON, A. (2005) Mechanisms of nutrient modulation of the immune response. J Allergy Clin Immunol 115, 1119-1128; quiz 1129

FERNANDES, G. (2008) Progress in nutritional immunology. Immunol Res 40, 244-261

FOX, C. J., HAMMERMAN, P. S. & THOMPSON, C. B. (2005) Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 5, 844-852

GOFF, J. P. (2006) Major advances in our understanding of nutritional influences on bovine health. J Dairy Sci 89, 1292-1301

GOOD, R. A., FERNANDES, G., YUNIS, E. J., COOPER, W. C., JOSE, D. C., KRAMER, T. R. & HANSEN, M. A. (1976) Nutritional deficiency, immunologic function, and disease. Am J Pathol 84, 599-614

GREINER, E. F., GUPPY, M. & BRAND, K. (1994) Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. J Biol Chem 269, 31484-31490

HOUDIJK, J. G. (2008) Influence of periparturient nutritional demand on resistance to parasites in livestock. Parasite Immunol 30, 113-121

JACOBS, S. R., HERMAN, C. E., MACIVER, N. J., WOFFORD, J. A., WIEMAN, H. L., HAMMEN, J. J. & RATHMELL, J. C. (2008) Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J Immunol 180, 4476-4486

JACOBS, S. R., MICHALEK, R. D. & RATHMELL, J. C. (2010) IL-7 is essential for homeostatic control of T cell metabolism in vivo. J Immunol 184, 3461-3469
JONES, R. G. & THOMPSON, C. B. (2007) Revving the engine: signal transduction fuels T cell activation. Immunity 27, 173-178
KAECH, S. M., HEMBY, S., KERSH, E. & AHMED, R. (2002) Molecular and functional profiling of memory CD8 T cell differentiation. Cell 111, 837-851
KEHRLI, M. E., JR. & GOFF, J. P. (1989) Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. J Dairy Sci 72, 1188-1196
KLASING, K. C. (2006) Negative consequences of immune responses: what can be done by nutritionists? Proc. Soc. Nutr. Physiol. 15, 17-23
LI, P., YIN, Y. L., LI, D., KIM, S. W. & WU, G. (2007) Amino acids and immune function. Br J Nutr 98, 237-252
MAGGINI, S., WINTERGERST, E. S., BEVERIDGE, S. & HORNIG, D. H. (2007) Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br J Nutr 98 Suppl 1, S29-35
MEHRZAD, J., DUCHATEAU, L., PYORALA, S. & BURVENICH, C. (2002) Blood and milk neutrophil chemiluminescence and viability in primiparous and pluriparous dairy cows during late pregnancy, around parturition and early lactation. J Dairy Sci 85, 3268-3276
MICHALEK, R. D. & RATHMELL, J. C. (2010) The metabolic life and times of a T-cell. Immunol Rev 236, 190-202
OHTSUKA, H., WATANABE, C., KOHIRUIMAKI, M., ANDO, T., WATANABE, D., MASUI, M., HAYASHI, T., ABE, R., KOIWA, M., SATO, S. & KAWAMURA, S. (2006) Comparison of two different nutritive conditions against the changes in peripheral blood mononuclear cells of periparturient dairy cows. J Vet Med Sci 68, 1161-1166
SAVINO, W. (2002) The thymus gland is a target in malnutrition. Eur J Clin Nutr 56 Suppl 3, S46-49
SCHAIBLE, U. E. & KAUFMANN, S. H. (2007) Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 4, e115
SCRIMSHAW, N. S. & SANGIOVANNI, J. P. (1997) Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 66, 464S-477S
SORDILLO, L. M., CONTRERAS, G. A. & AITKEN, S. L. (2009) Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim Health Res Rev 10, 53-63
STABEL, J. R., GOFF, J. P. & KIMURA, K. (2003) Effects of supplemental energy on metabolic and immune measurements in periparturient dairy cows with Johne's disease. J Dairy Sci 86, 3527-3535
TAMAS, P., HAWLEY, S. A., CLARKE, R. G., MUSTARD, K. J., GREEN, K., HARDIE, D. G. & CANTRELL, D. A. (2006) Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca2+ in T lymphocytes. J Exp Med 203, 1665-1670
WIEMAN, H. L., HORN, S. R., JACOBS, S. R., ALTMAN, B. J., KORNBLUTH, S. & RATHMELL, J. C. (2009) An essential role for the Glut1 PDZ-binding motif in growth factor regulation of Glut1 degradation and trafficking. Biochem J 418, 345-367
WOLOWCZUK, I., VERWAERDE, C., VILTART, O., DELANOYE, A., DELACRE, M., POT, B. & GRANGETTE, C. (2008) Feeding our immune system: impact on metabolism. Clin Dev Immunol 2008, 639803
WOODWARD, B. (1998) Protein, calories, and immune defenses. Nutr Rev 56, S84-92
YAQOOB, P. & CALDER, P. C. (2007) Fatty acids and immune function: new insights into mechanisms. Br J Nutr 98 Suppl 1, S41-45