Role of Leptin in Immunology

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Leptin seems to play an important role in the generation and maintenance of immune responses. Leptin is a cytokine similar in structure to interleukin 2, an important T-cell growth factor. Energy balance and supply is increasingly being realised as an important factor in the survival and function of immune cells. Immune responses are intrinsically energy expensive and come at a cost to the responding organism. A fall in leptin concentration, as occurs in starvation, causes impaired cellular immune responses with proinflammatory and Th1 immune responses being particularly affected. Animal models of leptin deficiency show impaired cognate immune responses and are resistant to a variety of autoimmune diseases, mainly those dependent on intact T-cell immunity. The next step is to determine whether modulation of the leptin axis is therapeutically beneficial in a variety of autoimmune or infectious diseases.

Key Words: leptin, immune system, interleukin 2, thymocytes, T-cell function, nutrition and infection

Leptin in Response to Nutritional Change

Many data suggest that leptin is important for optimal functioning of the immune system. We ought to think of leptin as a dynamically regulated cytokine with an evolutionary role in the emerging field of immunity and bioenergetics. In this context, leptin seems to effect direct peripheral effects on immune cells and indirect actions on these cells via the central nervous system.

Leptin is a helical cytokine and the only reason it is called a hormone is that an immunologist didn't discover it! It has four alpha helices and it is structurally, but not in sequence terms, very similar to interleukin-2 (IL-2), a

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fundamentally important T-cell growth factor. The leptin receptor also has amino acid motifs characteristic of type 1 cytokine receptors, such as those for granulocyte-macrophage colony-stimulating factor, IL-3, and leukemia inhibitory factor.

We have already heard a great deal about how serum leptin levels correlate with adipocyte mass and that a rapid reduction in serum levels is observed during fasting and starvation.

Equally importantly is the role of leptin in the systemic response to inflammation. It has been shown clearly in hamsters and mice that inflammatory stimuli such as lipopolysaccharide (LPS), IL-1, and tumor necrosis factor-α (TNF-α) can acutely raise leptin levels.[1–3] At the beginning of an immune response leptin levels rise very rapidly in a pattern typical of general cytokine gene induction and gene expression. The human clinical data in terms of leptin levels in disease states is not as clear, although there have been studies that show high leptin levels during sepsis, particularly when patients are severely ill in an intensive care unit. In these cases, leptin levels correlate very well with survival and also with circulating IL-6 levels.[4] There is therefore some evidence that leptin reacts in an appropriate physiologic fashion to help modulate an immune response early in the course of inflammatory response.

In a paper in *Nature,* Ahima[5] proposed that one of the important physiologic features of leptin was that a fall in leptin concentration acted as a signal of starvation and that if you gave leptin during starvation, you could blunt some of the neuroendocrine responses to starvation. Why should we be interested in starvation and immune responses? Research for many years has considered the energy requirements of the immune response and this is what links leptin and the immune system together. We now know that immune responses are very expensive in terms of energy.[6] We also know that as T-cells develop in the thymus, more than 95% of them die[7] so it is an enormously inefficient and expensive process, particularly in the cognate immune system where large-scale clonal expansion of T lymphocytes in response to environmental antigens is needed.

We also know that in humans involved in prolonged physical exercise there is an impairment of immune

responses in vitro.[8] There is also good evidence that immune responses or the capacity to mount an immune response can be traded off against energy requirements of other systems when energy is limited. Activation of the immune system can be costly in all species. So one of the questions is why would we want to down-regulate the immune response in times of starvation or relatively hypoleptinemic states? One might argue that this would be a relatively maladaptive process for combating the risks of infectious diseases. A paper by Moret et al.[9] gave physiologic proof of principle of the nutrition–immune system interaction. In a study in bumblebees, if you limited the nutrient intake of bees and activated the immune system, they would drop dead, whereas bees with unlimited nutrient intake survived. The conclusion from these experiments was that if your immune system takes energy in a time of limited energy, then that is sufficient to cause a three- or fourfold increase in mortality. From an evolutionary perspective one can imagine that there are situations in which it is worthwhile to down-regulate your immune response, such as in times of nutrient shortage.[10]

In human starvation, cell-mediated immune responses are down-regulated.[11] Antibody responses are reasonably well preserved but interferon-γ, which is very important in Th1 type proinflammatory immune responses, is very markedly suppressed.[12] So there is not just a global suppression of immune responses. Malnourished children are particularly affected by the sort of infectious diseases that are T-cell dependent[13,14] so pathogens such as measles, tuberculosis, and HIV are particularly serious. Vaccination efficiency, which is effectively a human in vivo experiment to look at T-cell priming, is also relatively poor.[15] The thymus gland where the T-cells develop has been called the barometer of nutrition. You can tell how malnourished a child is by looking at the size of the thymus, either at post-mortem or with ultrasound. We also know that the thymus, lymph nodes, and spleen undergo atrophy in malnutrition and that famine predisposes to infectious diseases.[16] We now even have suggestions of this happening in utero with programming of the immune response during times of famine.[17]

Animal Models of Leptin Deficiency

Long before leptin was discovered, the literature on the immune systems of *ob/ob* or *db/db* mice showed that skin graft rejection (a good in vivo marker of a T-cell-mediated response) was delayed.[18] As long ago as 1976 it was shown that if you infect *db/db* mice with Coxsackie virus the homozygous mice all died, whereas the wild-type mice all survived.[19] These mice have been described as having other defects in cell-mediated immunity, such as impaired interferon-γ production and T-cell proliferation. They also have thymic and lymphoid atrophy so they share some of the immune features of starvation.[20] With our current understanding of the role of leptin, how is this reflected in these genetically leptin-deficient mice? Have their immune systems developed at all? We have reassessed the thymus and confirmed that they have thymic atrophy with reduced numbers of naïve T-cells circulating in the blood.[21] If you challenge these mice with T-cell antigens to attempt to induce experimental autoimmune disease, they are completely resistant to this challenge. One particularly interesting paper showed that T-cell-mediated hepatitis could not be induced in *ob/ob* mice.[22] Nevertheless, if you administer leptin to these *ob/ob* mice then they do develop hepatitis. By contrast with the T-cell-mediated changes where marked deficiencies are apparent, when the innate immune system is tested to see whether septic shock occurs following macrophage activation, these leptin-deficient animals are hypersensitive to shock induced by both LPS and TNF-α. If normal mice are starved, thus inducing a typical fall in leptin, the sensitivity of these normal mice to septic shock increases; giving leptin can prevent this sensitivity during semistarvation.[23]

Experimental autoimmune encephalomyelitis (EAE), is a murine model for multiple sclerosis. This disease is Th1 T-cell dependent, which in turn means that interferon-γ is an important cytokine in the immune process leading to pathology. One group of *ob/ob* mice was treated with leptin at 1 $\mu g \cdot g^{-1} \cdot day^{-1}$ of initial body weight. A further group of these mice was injected with placebo and a third group was pair fed the same food intake of the leptin-treated group. The final two groups were wild-type congenic mice, treated with the same dose of leptin as above or saline. When injected with the immunogenic peptide in adjuvant, the normal mice became clinically sick and their condition was worsened considerably by the injection of leptin. The *ob/ob* mice, however, given placebo and either ad libitum or pair-fed intakes, were completely resistant to the induction of the EAE. When the *ob/ob* mice were then given leptin they became susceptible to induction of encephalomyelitis.[24,25]

Whether leptin is important in determining the survival of antigen-specific T-cells once they have been generated was tested by taking normal mice and injecting them with the stimuli needed to induce EAE. T-cells from these mice were taken and transferred 2.5×10^7 T-cells into the same test systems, i.e., animals with a defective *ob* gene or animals with a normal leptin system and an otherwise equivalent genetic background. We discovered that the presence of leptin—in either normal or *ob/ob* mice that were given leptin—is required for the persistence of these passively transferred T-cells and that the onset of the EAE is dependent on the persistence of these T-cells in the presence of leptin. In order to confirm

the clinical scoring, specific cytokine responses were analysed. Th1 responses are important for clearing viral and fungal infections and involve the generation of interferon-γ and IL-12. The Th2 system is antagonistic to the Th1 system and produces IL-4 so interferon-γ can be used as an index of Th1 activity and IL-4 responses as a reflection of Th2 activity. Again it is evident that only the *ob/ob* mice treated with leptin have any significant T-cell proliferation and as one increases the dose of the immunizing T-cell antigens only the *ob/ob* mice treated with leptin produce any interferon-γ, indicative of activation of the Th1 system. In these *ob/ob* mice treated with leptin there is no significant IL-4, indicative that there is no significant Th2 response. When one does not give leptin to the *ob/ob* mice then they can activate Th2 responses and one sees significant amounts of IL-4. Analogously, the wild-type mice have a normal proliferation of splenocytes whether or not they receive leptin. By contrast with the *ob/ob* mice, however, they have a modest response in both Th1 and Th2 systems unless they receive leptin when their interferon-γ (Th1) is amplified and their IL-4 (Th2) is suppressed.

We have extended these studies by considering the impact of starvation in normal mice, which have been sensitized with a T-cell-dependent antigen. Five or six days later we then rechallenged them with the DTP antigen and assessed the magnitude of the immune response. In our normally fed mice we find the expected delayed-type hypersensitivity (DTH) swelling response, but in starvation the immune response was profoundly suppressed. Then in a group given leptin at a dose of 1 µg/g of initial body weight every 12 hours for 48 hours in association with this starvation there was effectively full protection of the mice in terms their DTH response.[26] In addition, one can look at the thymus and assess the impact in starvation of providing leptin. Without leptin there is induction of cortical thymocyte apoptosis on starvation but by providing leptin at the same time, one can completely preserve the normal architecture of the thymus and limit apoptosis.[21]

One can attempt to take the same approach in humans, although this is more difficult. In two of Dr. O'Rahilly's patients with leptin deficiency we have attempted to assess the response to a polyclonal mitogen in terms of the proliferation and cytokine production of the T-cells. We found, in these limited preliminary experiments, that in the absence of leptin, T-cell proliferation and cytokine production (particularly interferon-γ) were markedly suppressed when compared with normal controls. Leptin therapy restored these responses to approximately normal and in some cases to levels equal to controls. This evidence implies that one is getting comparable modulation of the T-cell responses to leptin in humans and in animals. Clearly we do not know whether

in the human these responses depend on changes in the hypothalamic pituitary axis with changes in corticosteroid production or whether there are other metabolic effects in addition to the leptin changes. However, it does seem as if these human cases do not display the abnormalities of the corticosteroid axis observed in *ob/ob* mice.[27]

We have shown that leptin has both direct and indirect effects on the thymus with leptin also being important for the proliferation and survival of T-cells. Once naïve, i.e., non-antigen-stimulated T-cells have passed from the thymus to the lymph node, they then on exposure to an antigen, proliferate, and secrete cytokines of characteristic of either a Th1 or Th2 phenotype. We have also shown that leptin tends to skew the response to a Th1 or proinflammatory state, whereas with low or absent leptin the Th2 immune response dominates.

In conclusion, it is well established that the adipocyte-derived protein leptin plays a major role in the regulation of energy homeostasis. There is now increasing evidence that leptin also plays an important role in immune responses and that energy balance per se is a critical aspect of immunity.[28] Leptin has been shown to have specific effects on innate and adaptive immune responses both in vitro and in vivo and polarizes T-cells toward a Th1 phenotype. Mice deficient in leptin or its receptor are immunodeficient and are resistant to the induction of a variety of immunologically mediated diseases. Targeting pathways involved in cellular energy balance, such as the leptin axis, may prove to be therapeutically beneficial in a variety of immune-mediated conditions.

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