Beef Session III

Cow-Calf/Feedlot Combined

Gary Oetzel, *Presiding*

The Role of Interleukin 2 in the Immune Response of Incoming Feeder Cattle

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The cattle industry is very important to the agricultural economy of the United States. The Midwest, in general, and Kansas, in particular, rely heavily on the cattle-feeding industry for a strong healthy economy. However, the cattle industry is suffering a severe cost/price squeeze. Some of the economic difficulties that exist in animal agriculture arise from costly production losses especially those caused by production diseases, such as bovine respiratory disease. Although numerous experiments have and will continue to be conducted on the nature of infectious agents involved in bovine respiratory disease, complete protection from the disease by bacterins, vaccines, and chemoprophylactic agents has not been achieved. Current thinking on the etiology of many production diseases suggests that the homeostatic stress responses in the animal are important contributing factors to disease susceptibility. Solutions to the problems of production diseases will be found only when a better understanding of resistance and cellular messenger mechanisms is available to modify production methods.

Stress and Disease

There is now a clear relationship between stress and many diseases. It has been known for many years that minimizing environmental stressors is important for sound animal management. As of yet, however, it is not clear exactly how stressors alter host resistance to disease. Any of a variety of stressors may act as a trigger which sets the infectious agents on their pathogenic course. Stressors during shipment, such as feed and water deprivation, fatigue, chilling, anxiety, and exertion, may predispose animals to the actions of pathogens encountered by direct contact from transporting vehicles or assembly areas, or more likely, to the actions of pathogens that are continually present in the respiratory system in low numbers (1, 2). If immunopotentiating regimes for stressed calves are to hold any promise, then a better understanding of the basic cellular immune processes involved must be gained.

Published as contribution 86-210-J, Kansas Agricultural Experiment Station.

Glucocorticoids

The importance of the hypothalamus-pituitary-adrenal axis in stress and disease has received much attention since it was first proposed (3). For example, reactivation of latent infectious bovine rhinotracheitis virus (IBRV) is thought to be caused by stress-induced increase in corticosteroid levels (4). Pharmacologic concentrations of dexamethasone administered *in vivo* have been shown to suppress lymphocyte blastogenesis and polymorphonuclear leukocyte function in cattle (5, 6). We have found that acute physical stress increased plasma cortisol concentration five-fold, decreased cellular immune function, and increased IBRV replication in feeder calves (7). Similarly, adrenocorticotropic hormone (ACTH) has been shown to impair lymphocyte blastogenesis when administered to cattle over a 3-day period (8). Collectively, these data suggest that high levels of glucocorticoids impair immune function in cattle. However, this suggestion assumes that all of the stress-induced alterations in immune function are caused by elevated glucocorticoids; that assumption may not be correct (9-11). Whether transport or other management-induced increases in cortisol are correlated with immunosuppression in cattle is not clear.

Glucocorticoids and Immune Regulation

The physiological and immunological mechanisms responsible for stress-induced impairment in immune responsiveness are unknown (12). The generation of an immune response requires not only interaction between cells of mononuclear and lymphoid lineages but also the release

of appropriate cytokines by these cell populations (13, 14). Interleukin 1 (IL 1) is a monokine that is released by activated macrophages and is considered to be essential for T cell activation (15). The lymphokine, interleukin 2 (IL 2) is released by stimulated T cells and is required for clonal expansion of T lymphocytes (16, 17). Recent reports (18-20) indicate that IL 2 receptors are also expressed on B lymphocytes. Similar to most ligand-receptor interactions, the specificity of the immune system is maintained at the level of induction and expression of cellular receptors (21-22). Defects in production and responsiveness to IL 2 have been observed in many immune deficiency states (23-27). Significantly, IL 2 has also been shown to be involved in immune interferon regulation (28). These examples illustrate the important regulatory role that interleukins play in T cell proliferation and the immunological problems that can occur when defects in these regulatory pathways ensue.

Do stressful environments induce physiological changes in animals that alter interleukin production or responsiveness? Unfortunately, the answer to this question is unknown. However, results obtained from *in vitro* studies evaluating the influence of dexamethasone on IL 2 production indicate that high levels of plasma cortisol may decrease IL 2 production (29, 30). The hypothesis that IL 2 may be decreased by stress-induced elevations in plasma cortisol is further strengthened by data that have shown lower concanavalin A-induced blastogenesis of bovine and porcine lymphocytes when cultured with high physiologically attainable levels of cortisol (31, 32). These findings suggest that environmental and management stressors in feeder calves may lower immune reactions by altering IL 2 production or activitiy *in vivo*. If this hypothesis is correct, then methods of modulating lymphokine or monokine metabolism or therapy with purified cytokines or biological response modifiers may benefit immunosuppressed animals.

We have conducted several experiments that link stressful events imposed on feeder calves to impaired immune function (7, 33, 34). However, we have just begun to explore the molecular mechanisms involved in stress-induced immunosuppression in cattle (35). Data from our *in vitro* studies indicate that cortisol at physiologically attainable concentrations lowers IL 2 production and that increasing concentrations of cortisol are correlated with both lower lymphocyte proliferative responses and lower IL 2 activity. The results of a recent experiment indicate that lymphocytes from calves injected with adrenocorticotropic hormone (ACTH), in addition to displaying lower lymphocyte proliferative responses, produce less IL 2 than saline-injected controls (36). These results lead us to believe that our hypothesis of stress-induced immune alterations via lymphokine regulation may be correct. However, we are cautious not to extrapolate these data beyond the scope of the experimental design, since natural stressors of cattle, such as shipment and physical exertion, have not yet been evaluated.

Conclusion

Currently, there is much interest in finding a means of modulating the immune response in stressed calves. Various immunopotentiating drugs (Levamisole) and biologics (interferons) have been evaluated as a means of alleviating the high morbidity of bovine respiratory disease. In fact, many of the new biotechnology companies are investing substantial amounts of capital in research and development projects aimed at pursuing the use and application of bovine interferon in the cattle industry. Other companies are actively investigating the regulation of interleukin metabolism in stressed and/or diseased cattle. Those experimental approaches to the problem of bovine respiratory disease have merit and will be continued by many investigators. However, if solutions to the problem of bovine respiratory disease are to come via immunomodulation, then it is imperative that a better understanding of the cell regulatory mechanisms in stressed calves be gained.

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Questions & Answers:

Question: What products are now available?

Answer: Human IL-2 is available commercially. Bovine recombinant IL2 is available commercially. There are a variety of ways to attack this problem. This afternoon Dr. Cummins will speak on interferon. A variety of experiments and emphasis is going into interferon, which I think should be. And that's certainly an area that is offering some promise. As a group of scientists and companies are looking at interferon, there are also companies and scientists that are looking at interleukin-2. Part of the problem with interleukin-2, as with a lot of drugs, is delivery systems. This protein has a half-life of about 3-6 minutes. What is it going to do? Clearly it's not going to be a magic bullet that's going to turn everything around. It's obviously going to be effective where we have defects in IL2 metabolism, but that may not occur in every situation. As I was talking earlier with some in the audience, interleukin-2 in a purified state in a lot of volume, may have a place in terms of adjuvant type of effect with vaccination of animals coming into the feedlot when vaccinated in conjunction with IL2 that may have some benefit. There have been some preliminary studies that have shown some benefit from that type of event.

Question: Has anyone administered IL2 to animals?

Answer: Yes. I'm not sure about cattle. I'm sure it has probably happened. But I do know human interleukin-2 is being pumped into pigs right now. We're assaying samples from those animals. But there are many of those studies that have already been done in humans and laboratory animals, and they don't always show a benefit.

Question: Is this the same as T-cell growth factor?

Answer: Yes, T-cell growth factor was the name that was used previous to 1979 or '80, and then they confused the issue with interleukin.

Question: The cortisol levels that are precipitated by implants, are they involved in what we may see?

Answer: I don't know. Certainly if cortisol goes high enough there may be. There is some early data that shows actually a beneficial effect of some implants in stress situations, such as heat stress, or with Ralgro. I think one of the things that we see, and I have had clinicians and practitioners relate this to me many times, is that when I show the dexamethasone data in feedlot heifers where dexamethasone or other glucocorticoids are used, often times what we see is an outbreak in those animals that have been treated with dexamethasone to abort them. Clearly I think one of the mechanisms involved in there is immunosuppression. What would happen if at the same time we gave those animals something to abort them—gave them IL2 or some other drug? These are areas that have promise I think, but we really don't know anything about.

Question: What cell lines do you use for IL2 assays and what about species specificity of IL2-?

Answer: The way IL2 assays is by a bio-assay. So we use a cell line. For the bovine we use a bovine cell line that we have generated and keep growing in culture continuously. We also assay for porcine on IL2. There we have a mouse cell line that we obtained from France that is a murine cell line, but pig IL2 works fine on it. Bovine doesn't. There is species specificity in IL2 and it seems to go, although there are exceptions, it seems to go. Man IL2 doesn't work too well on bovine cells and bovine doesn't work on mouse cells very well. So there is certainly species specificity.

Question: Why do you use 48 hours instead of 72 hours for the blastogenesis assay?

Answer: We have set those up for the different species that we use and those seem to provide us the optimal responses. There is a 48-hour incubation before treatment and then there is another 18 hours. So we are looking at a total time of 66 hours of incubation.