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Role of Biothera's APG™ 3-6 in Animal Health and Performance.

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The purpose of this paper is to provide an overview of the role of APG™ 3-6, a purified yeast derived 1,3 – 1,6 β-glucan in supporting health and performance of animals. Biothera yeast β-glucans have been reported as potent immunomodulators for various severe infectious diseases including anthrax[i, ii, iii, iv, v, vi, vii, viii]. Biothera's products also enjoy a broad patent protection applicable to animal health[ix].

The immune-enhancing activity associated with yeast β-glucan is derived from priming the innate immune system of animals (defined in the section on mode of action). It is critically important to note that yeast β-glucan functions by leveraging both the innate and acquired immune systems to combat infections. This topic will be discussed and defined in the section of this paper entitled "Mode of Action", it is one of a set of critical recent discoveries related to yeast β-glucan that will be presented for discussion in this paper.

Mode of action

It has been shown that macrophages and neutrophils, the most abundant cells of the innate immune system, play an important role in the mechanism of action of Biothera yeast β-glucans. We now know that the CR3 receptor is key to the role of Biothera's yeast β-glucans in immune modulation.

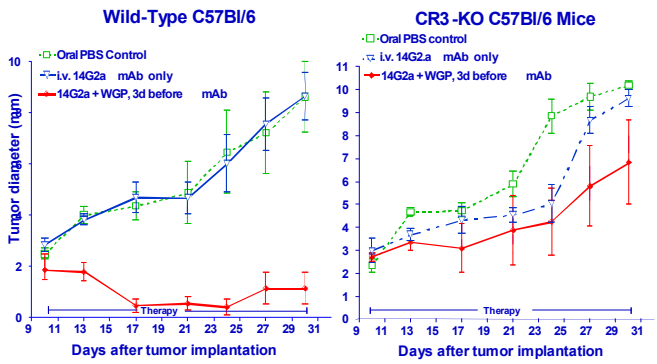
There are two principle forms of Biothera's yeast β-glucan that are absorbed by macrophages as the starting point for the mode of action, soluble glucan and whole glucan particles. Soluble glucan molecules are delivered by injection (i.v.) and engulfed by macrophage cells. Orally consumed whole glucan particles are uptaken through Peyer's Patches in the small intestine (Figures 1 and 2) and subsequently engulfed by macrophages.

The Peyer's Patches are a specialized immune tissue in the intestine that sample ingested material to prepare the body for potential pathogenic challenges. It has been shown that yeast β-glucan that is consumed with the diet is recognized as a carbohydrate that is present on pathogens but not animal cells and consequently "sampled". Hong et al.[x] demonstrated that at the Peyer's patch, M-cells transport yeast β-glucan across the epithelial lining of the small intestine and deliver the material to macrophage cells. The macrophage cells ingest the glucan particle through the process of phagocytosis. Macrophage cells transport the engulfed glucan particle to the various immune organs of the lymphatic system including, bone marrow, spleen and thymus. The glucan particle is digested by the macrophage over a period of days at these sites.

The smaller soluble, macrophage-digested β-glucan (fragment) is the bioactive form. It is released by the macrophage and binds to an abundant species of white blood cells known as neutrophils. Neutrophils are the key immune cell for the clearing of pathogenic cells. These neutrophils are now "primed" to respond to an immune challenge such as a bacterial infection. This leads to the next step in understanding the mode of action, the role of the primed neutrophils and antibodies.

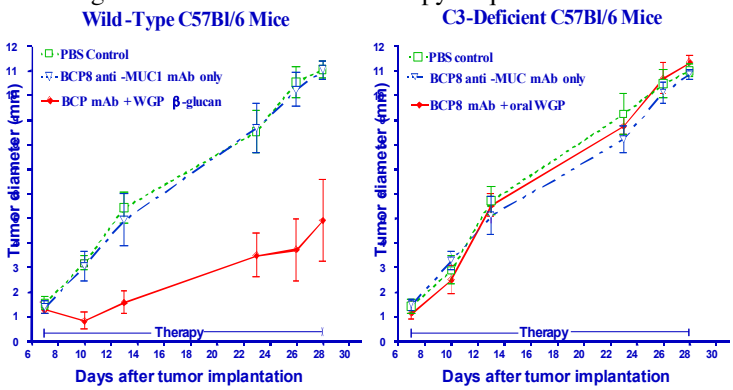
Neutrophils have receptors on their cell surface that help them interact with their environment. The specific receptor of interest to the present mode of action is the complement receptor 3 (CR3)[xi, xii]. Figure 3 (WGP® 3-6 is the human counterpart of APG 3-6) shows that in the presence of the CR3 receptor, β-glucan binds to the receptor along with a fragment of the C3 complement protein (requires a complement activated antibody, here 14G2A mAb). One key observation is that when the CR3 receptor is not present (CR3-KO knockout mice, Figure 3), the antibody-β-glucan treatment has no effect on cancerous cells (there was a 20% survival rate with β-glucan). An analogous mechanism exists for the CR-3 receptor and antibodies produced in response to infectious diseases.

Figure 3. WGP® 3-6 Activity in Cancer is CR3 -Dependent



During an infectious event antibodies bind to antigens on the surface of a bacterial cell and activate the binding of complement 3 (C3, opsonization). As the name suggests, this is the way by which the neutrophils recognizes and bind to an invading pathogenic cell via their C3 receptor (CR3). The CR3 receptor has two binding sites, one site for C3 on the pathogenic cell and one for the β-glucan fragment. The C3 site is essential for binding to pathogenic cells and the fragment specific site is essential for activating the killing mechanism to eliminate pathogenic cells. (Figure 4) [10] These studies were done in cancer models, but a similar mechanism applies to microbial pathogens. Thus, by adding Biothera's yeast β-glucan to the immune system through, for example, dietary supplementation the immune system is "primed" for action to more rapidly clear the system of infectious agents.

Figure 4. Oral WGP ® 3-6 Therapy Requires Serum C3



Results of animal efficacy studies
To date there have been a few immune biomarker and performance studies conducted with Biothera's APG 3-6 product. These studies have been performed on piglets, calves, and laboratory animal models.

Recent piglet trial with APG 3-6
In a recently conducted performance study 1200 piglets were distributed into two treatment groups of 600 animals; one received APG 3-6 and one served as the control group. Treatment and control animals were housed in separate rooms in the same barn with 40 animals per group, ear-tagged according to weight (cutoff weight was 9 lb. (4 kg)). Piglets were further separated according to normal and light weight piglets. Treatment animals received 0.075% PG 3-6 during the first 14 days post-weaning. The effect of Biothera's APG 3-6 health additive was evaluated average daily gain, total weight gain and mortality.[xiii]

Results of the study demonstrated an increase in ADG and total weight gain for animals receiving APG 3-6. A lower mortality was observed in all APG 3-6 treatment groups compared to control groups, but was not statistically significant.

Impact of APG 3-6 used in Calf Milk-Replacer Diets
Several university and private commercial studied have evaluated the benefits of APG 3-6 on performance of calves receiving milk replacer. A critically importance aspect of calf management is the early feeding regimen that typically depends on quality milk replacer to maintain animal health. In several studies completed by the USDA-ARS and at Purdue University in the U.S., APG 3-6 has been demonstrated to increase general immune health as part of a diet that includes milk replacer.

In one study researchers reported the following: "The objective of this study was to examine the innate immune response in peripheral blood and tissues, after 21 d of oral feeding of beta-glucan with or without ascorbic acid" … "Results indicate that feeding beta-glucan results in an up regulation of innate cell surface proteins associated with immune activation, and is further modulated by ascorbic acid."[xiv]

A second study[xv] by the same research group measured the impact of various immune biomarkers. The general observation was that APG 3-6 improved biomarkers that are related to the immune health of milk-replacer fed calves.

It is important to note that a leading producer of milk-replacer products in the U.S. has recently launched a major product containing Biothera's APG 3-6. Our U.S. partner has conducted multiple animal studies at their research facilities and concluded that APG 3-6 provides health and growth performance benefits

(results to date are unpublished and confidential)

Synergy of Biothera's APG 3-6 with other animal health products
There have been many additional studies animal models that further support the efficacy of Biothera's β-glucan in combating various infectious diseases.

Research conducted on the synergy of β-glucan with antibiotics has demonstrated a strong synergism between antibiotics and Biothera's β-Glucan[5]. In one study (Figure 6) it was demonstrated that the synergistic combination of β-glucan and a common antibiotic increase the ability of guinea pigs to resist septic infection by antibiotic resistant bacteria. An alternative interpretation of the data suggests that use of APG 3-6 allows for the use of lower doses of antibiotic to maintain animal health.

Biothera's β-glucans in infectious disease models
In another study[xvi], APG 3-6 was administered to mice that were subsequently infected with high doses of pathogenic bacteria (E. coli and S. aureus). Because the β-glucan acts synergistically with antibodies (see mode of action), a septic infection was prevented in the treatment group vs. the control group, all of which died of bacterial sepsis (figure 7).

Anthrax is a deadly disease that affects both animals and humans. In a study conducted by the Canadian Department of Defense, Dr. Kournikakis showed that orally administered APG 3-6 protected mice against anthrax infection[3]. A dose of Biothera's oral whole glucan particles (2 mg/kg body weight or 20 mg/kg body weight) for eight days prior to infection with *Bacillus anthracis* protected mice against anthrax infection over the 10-day post-exposure test period. Mice treated with antibiotic alone or β-glucan alone did not survive.

A second experiment was conducted to investigate the effect of orally-adminstered yeast ß -glucan after exposure of mice to *B. anthracis*. The results were similar to the previous experiment with an 80-90% survival rate for mice treated with ß-glucan, but only 30% for the control group after 10-days of exposure. Of course, the hopeful inference is that similar results would be observed with humans.

Conclusion

The use of APG 3-6 as an immunomodulator in animal health has a sound scientific basis. Additional studies are continuing to define the optimal role of APG 3-6 in both animal performance and for combating infectious diseases. We understand the mechanism of action, have researched the role of APG 3-6 in combating infectious disease and continue to develop research data to support the efficacy of APG 3-6 in animal health.

References

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[ix] U.S Patent 5,702,719
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U.S Patent 5,741,495
U.S Patent 5,504,079
U.S. Patent 5,849,720
U.S. Patent 5,622,940
U.S. Patent 5,032,401
U.S. Patent 5,322,841

Note: this is a small relevant selection of the overall patent portfolio pertaining to Biothera's beta-glucan technology

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[xv] Eicher, S.D. and D. C. 2005. Modulation of Toll-like Receptors 2 and 4 and Interleukin-1 RNA expression of blood leukocytes by beta-glucan and ascorbic acid in Neonatal Calves., Eicher, S.D. et al., 2005. 38th annual meeting of Society of Leukocyte Biology, Abstract 162.

[xvi] Biothera unpublished results (acquired from Alpha Beta Technology Inc.)