Functional ingredients: How to evaluate them?

Vaclav Vetvicka, PhD, Professor of Pathology at the University of Louisville

Possible ways

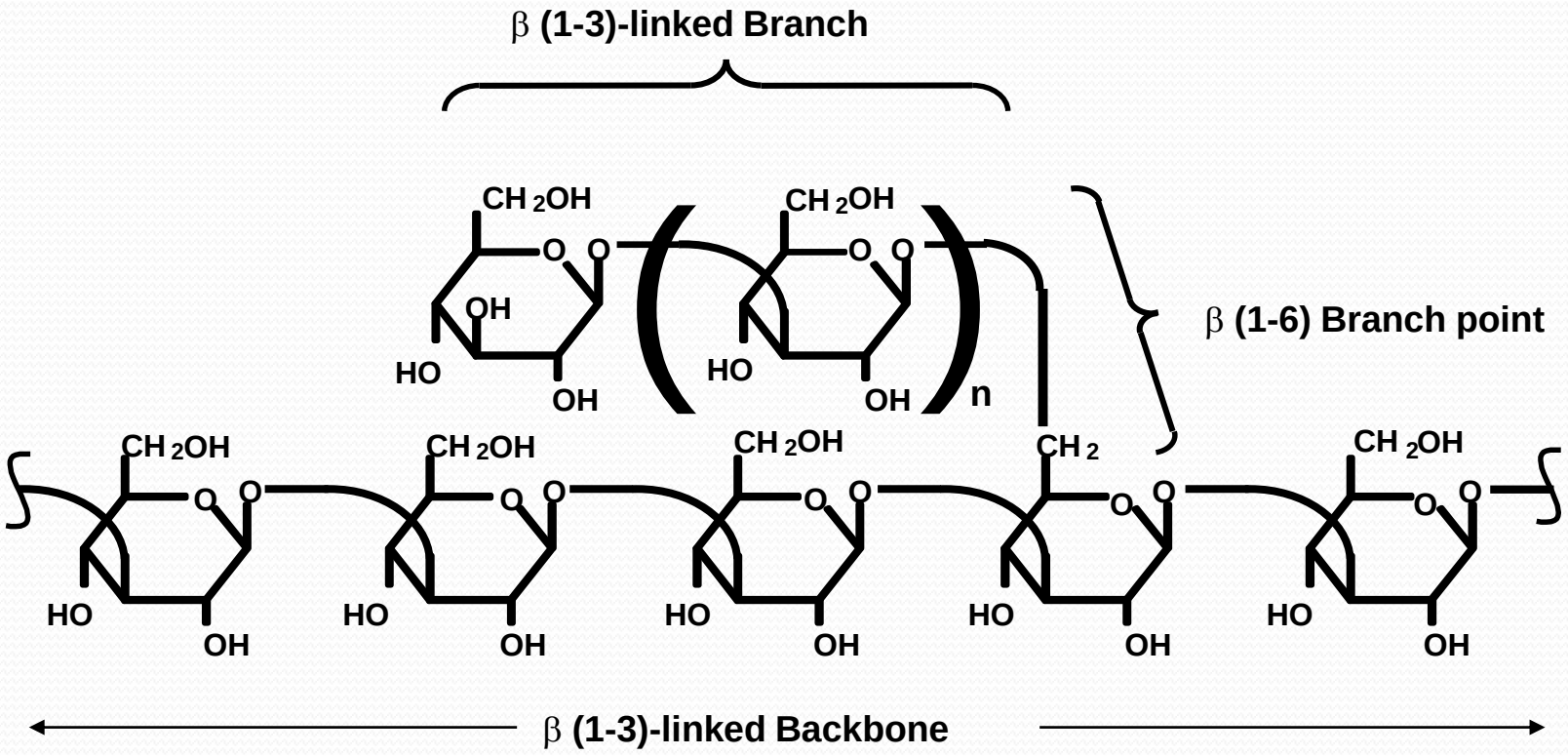
- ***In vitro* experiments**
- ***In vivo* experiments**
- **Clinical trials**

Glucan can serve as an example

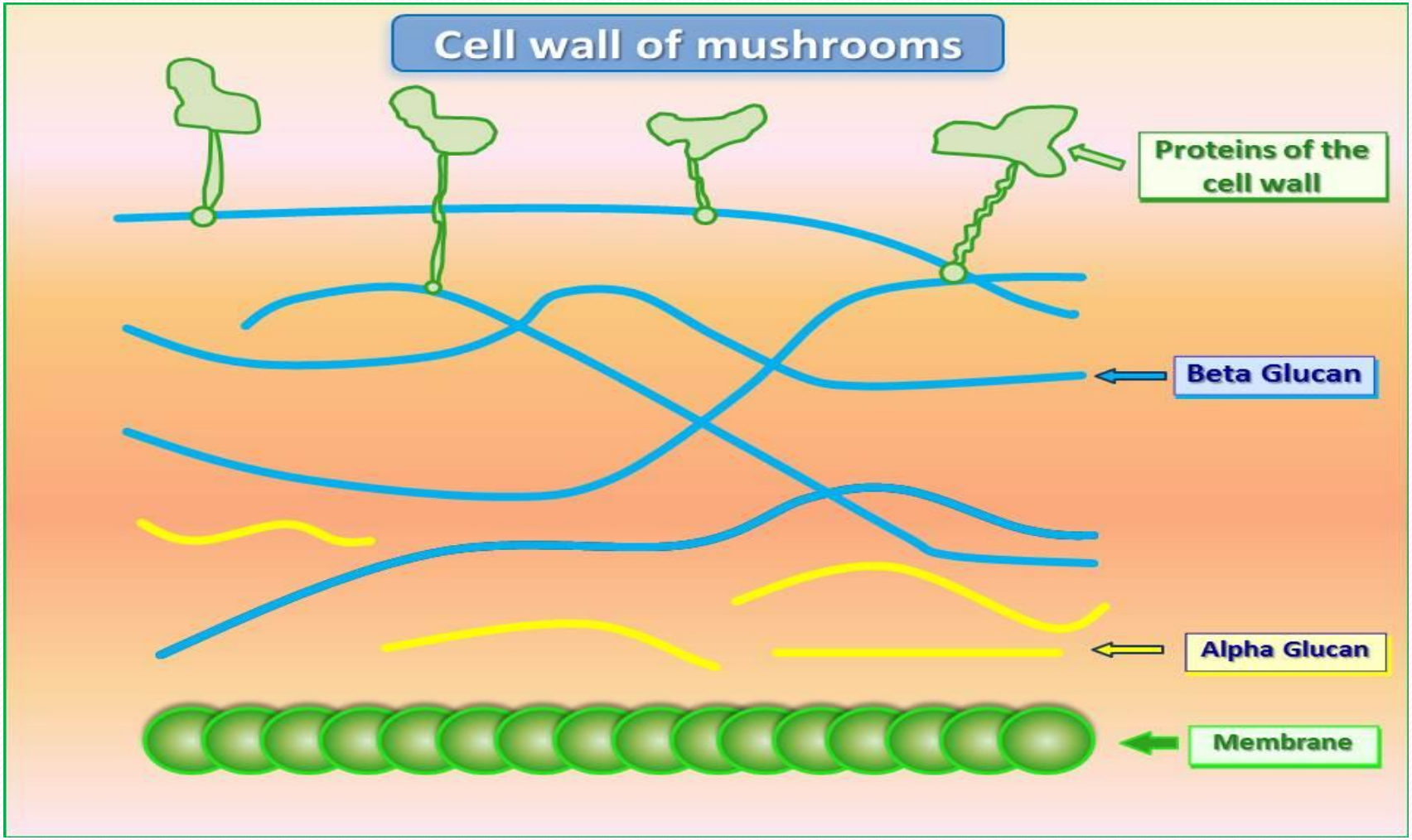
What is glucan?

- Natural polysaccharide, existing in numerous molecules of glucose bound together in several types of linkages
- Natural immunomodulator (sometimes called biological response-modifier)
- Exists in either soluble or insoluble form

Chemical structure



Glucan in cell wall



Possible sources of glucan

- Yeast (*Saccharomyces cerevisiae*)
- Mushrooms
- Bacteria
- Seaweed
- Cereal grains (oats)

Sensitive species

- Bees
- Shrimps, fish
- Mice, rats, guinea pigs, rabbits, cats, dogs
- Chicken
- Pigs, horses, cows
- Apes
- Humans

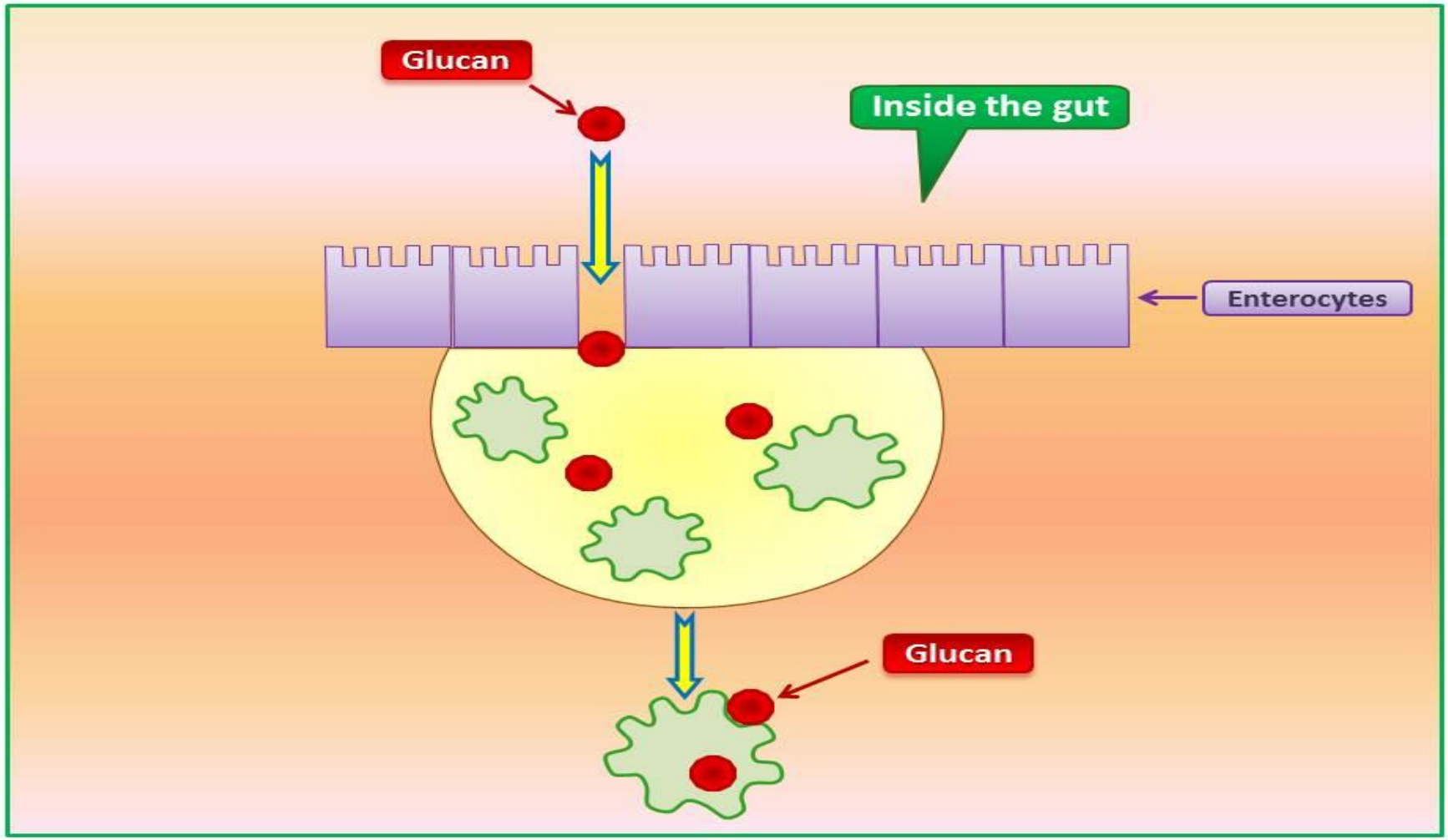
Known effects

- Nonspecific stimulation of defense reactions
- Anti-infection immunity
- Anti-cancer immunity
- Wound healing
- Lowering of cholesterol
- Stress reduction

Known effects (Cont.)

- Support of myelopoiesis
- Non - specific stimulation of defense reactions
- Anti-irradiation protection
- Adjuvant effects
- Modulation of blood sugar

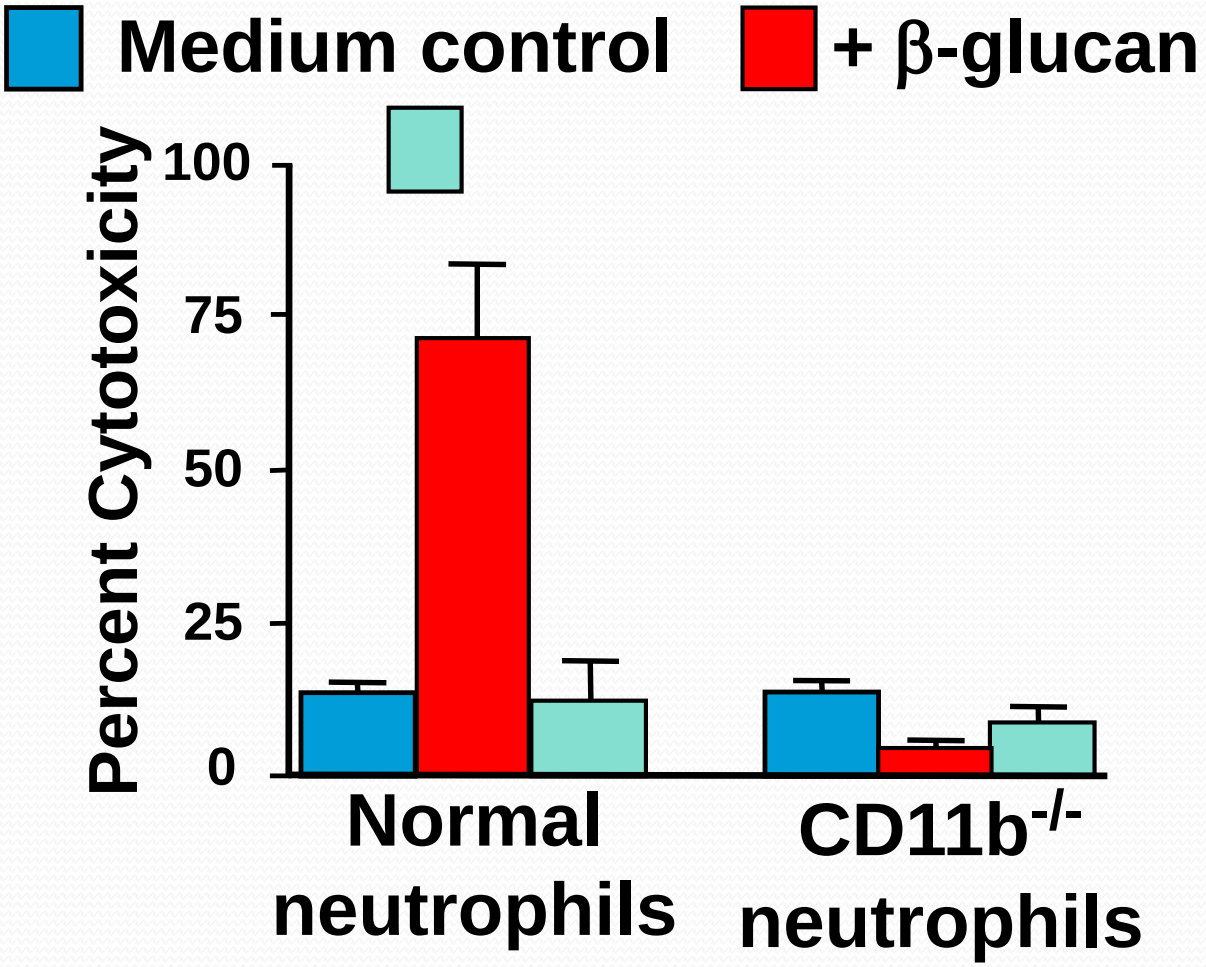
Glucan enters our body



Specific receptors

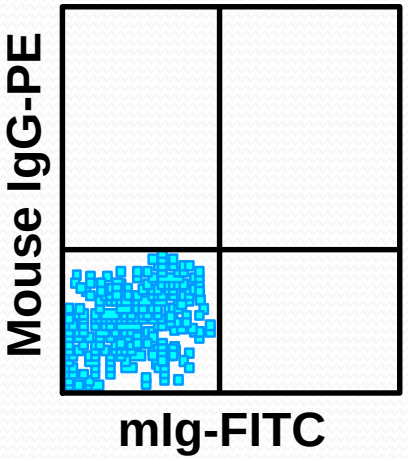
- The most important are:
- Complement receptor 3 (CR3)
- Dectin-1

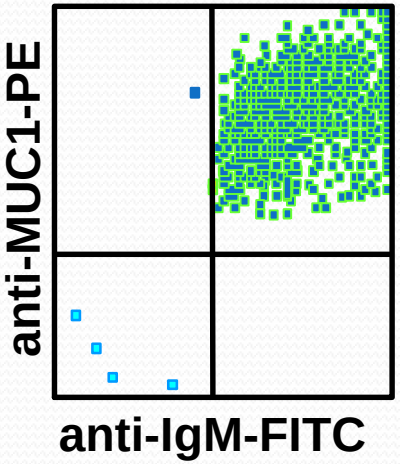
β-Glucan Priming of Murine Neutrophil CR3 Allows Subsequent Cytotoxic Triggering by iC3b-Opsonized Breast Tumor Cells



Two-Color Flow Cytometry Analysis of Tumor Cells from a Patient with Mammary Carcinoma for IgM, IgG, or C3

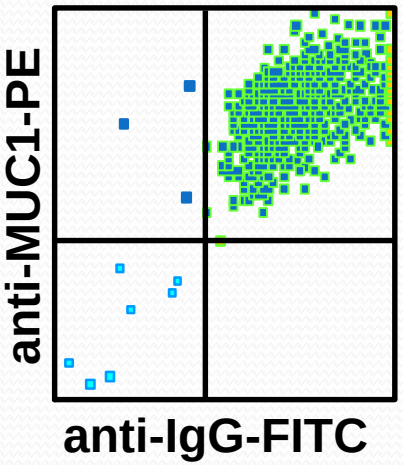
Negative control staining

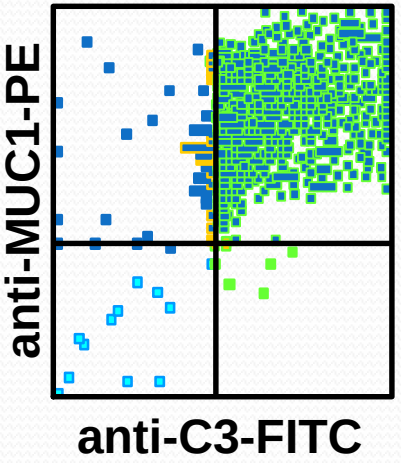




All MUC1⁺ cells have membrane bound IgM Abs

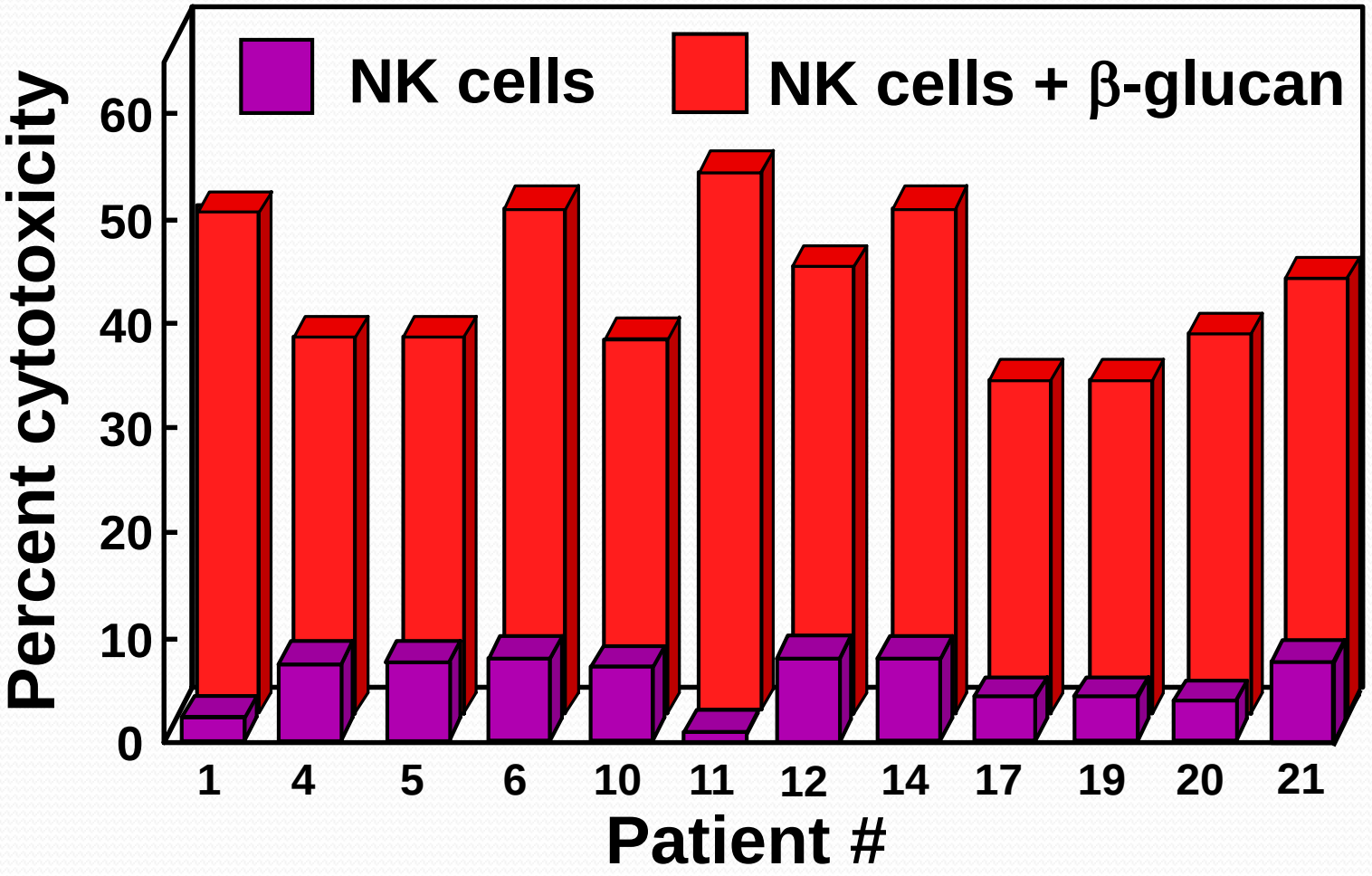
All MUC1⁺ cells have membrane bound IgG Abs



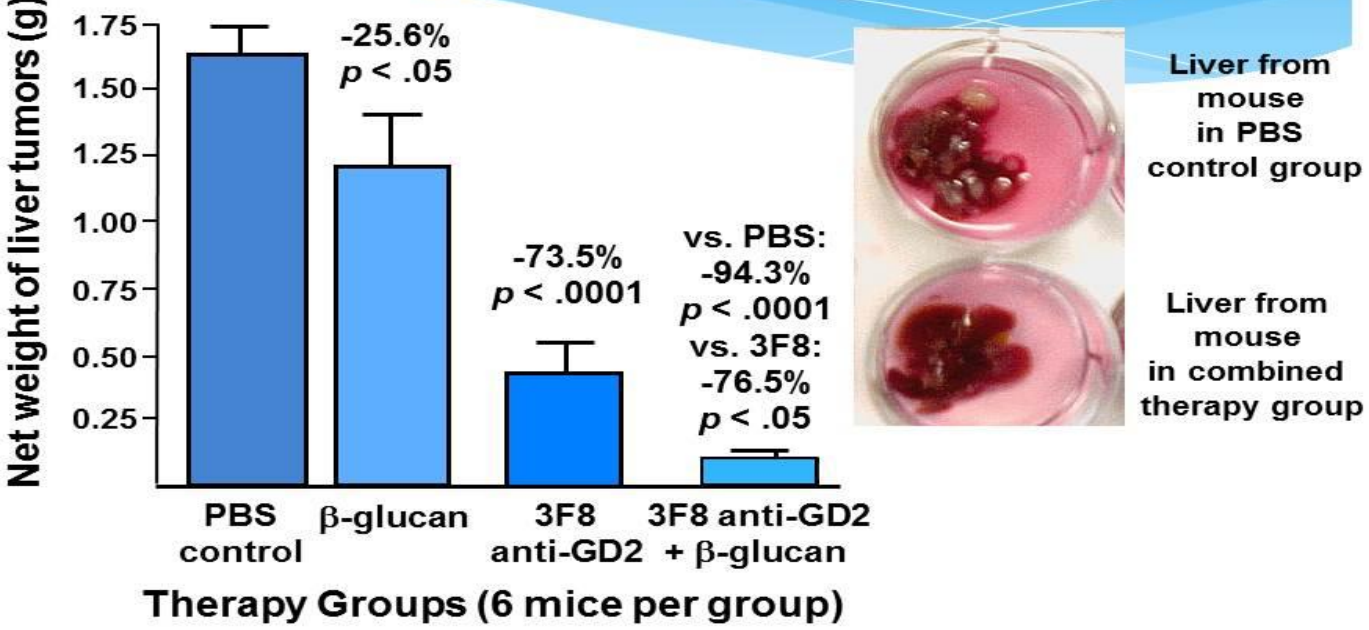


>90% of MUC1⁺ cells have membrane bound C3

Suspensions of Freshly Excised Primary Mammary Tumor Cells Bear Sufficient C3 for Cytotoxic Recognition by Allogeneic NK Cells Bearing β-Glucan-Primed CR3

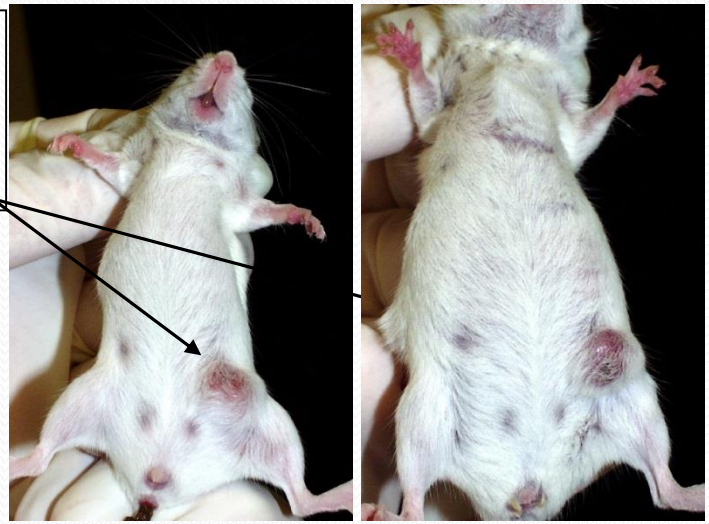


Glucan and liver cancer



Oral Glucan Functions in Mammary Tumor Reduction in Combination with Anti-Tumor mAb Nearly as Well as I.V. Soluble Glucan

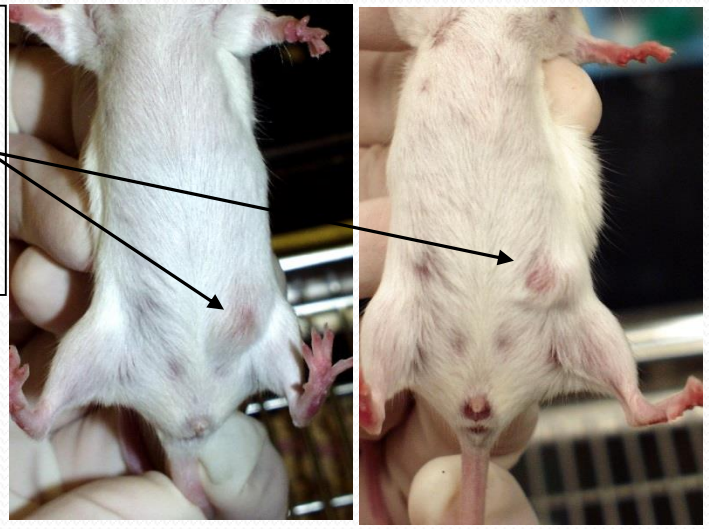
Mice in control group receiving i.v. mAb 11C1 only; tumors are ~9 mm diameter



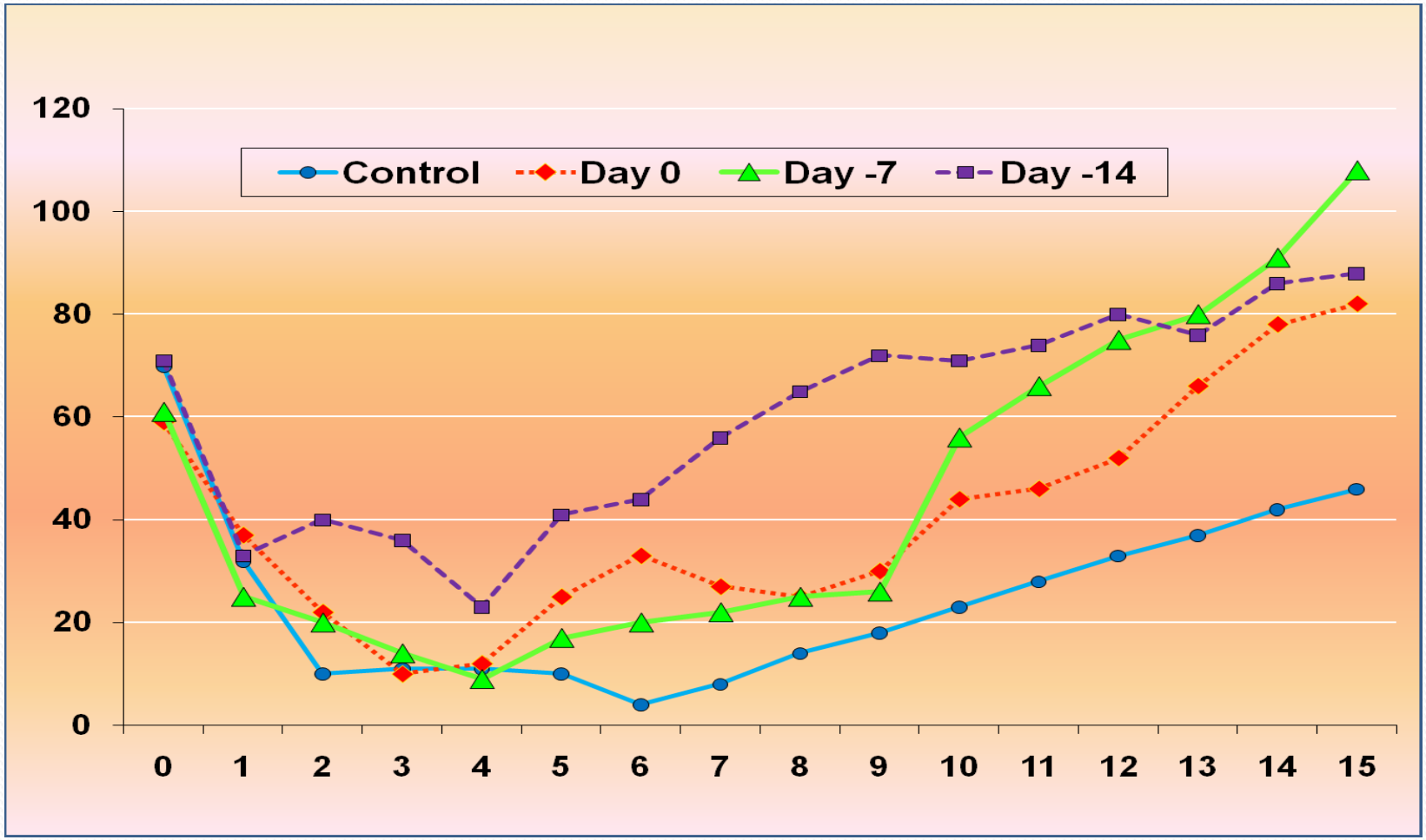
Mice receiving i.v. mAb 11C1 plus i.v. soluble β-glucan (NSG); 4 out of 5 mice have No visible tumor.



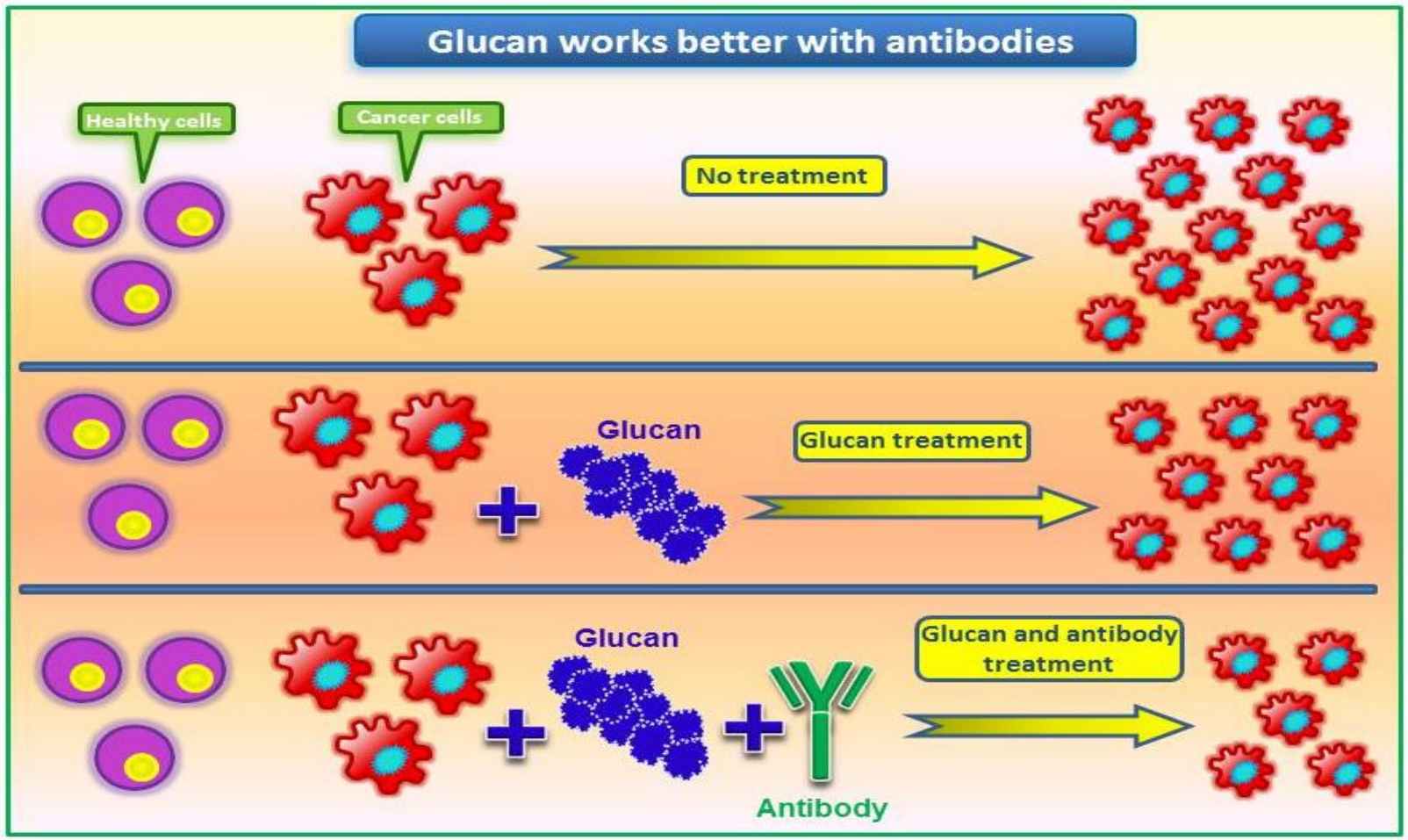
Mice receiving i.v. mAb 11C1 plus oral WPG, 200 μg/day; tumors ~20% of control; 2 out of 5 mice have barely visible tumors



Glucan-induced recovery of bone marrow after irradiation



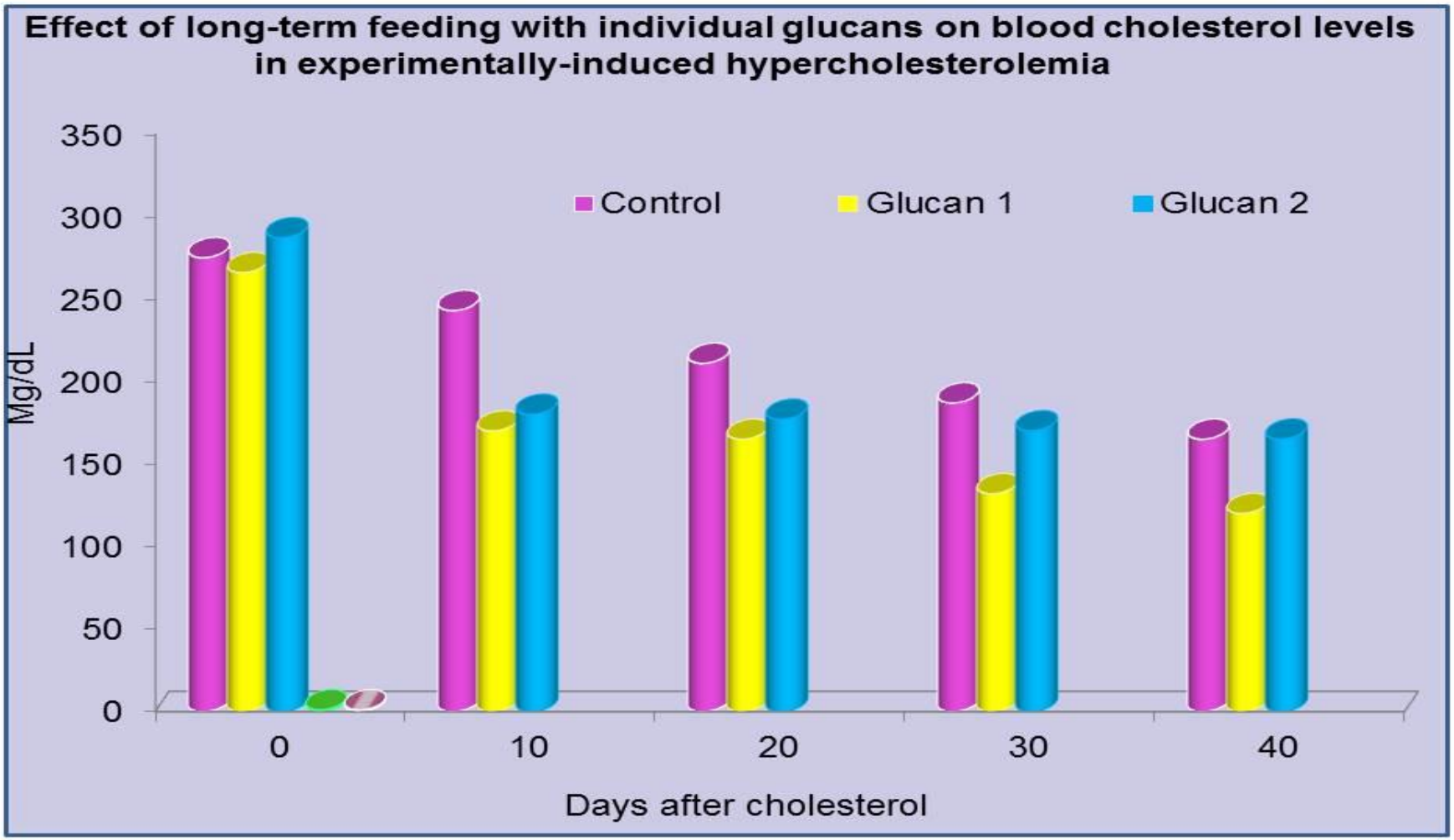
Glucan and antibodies



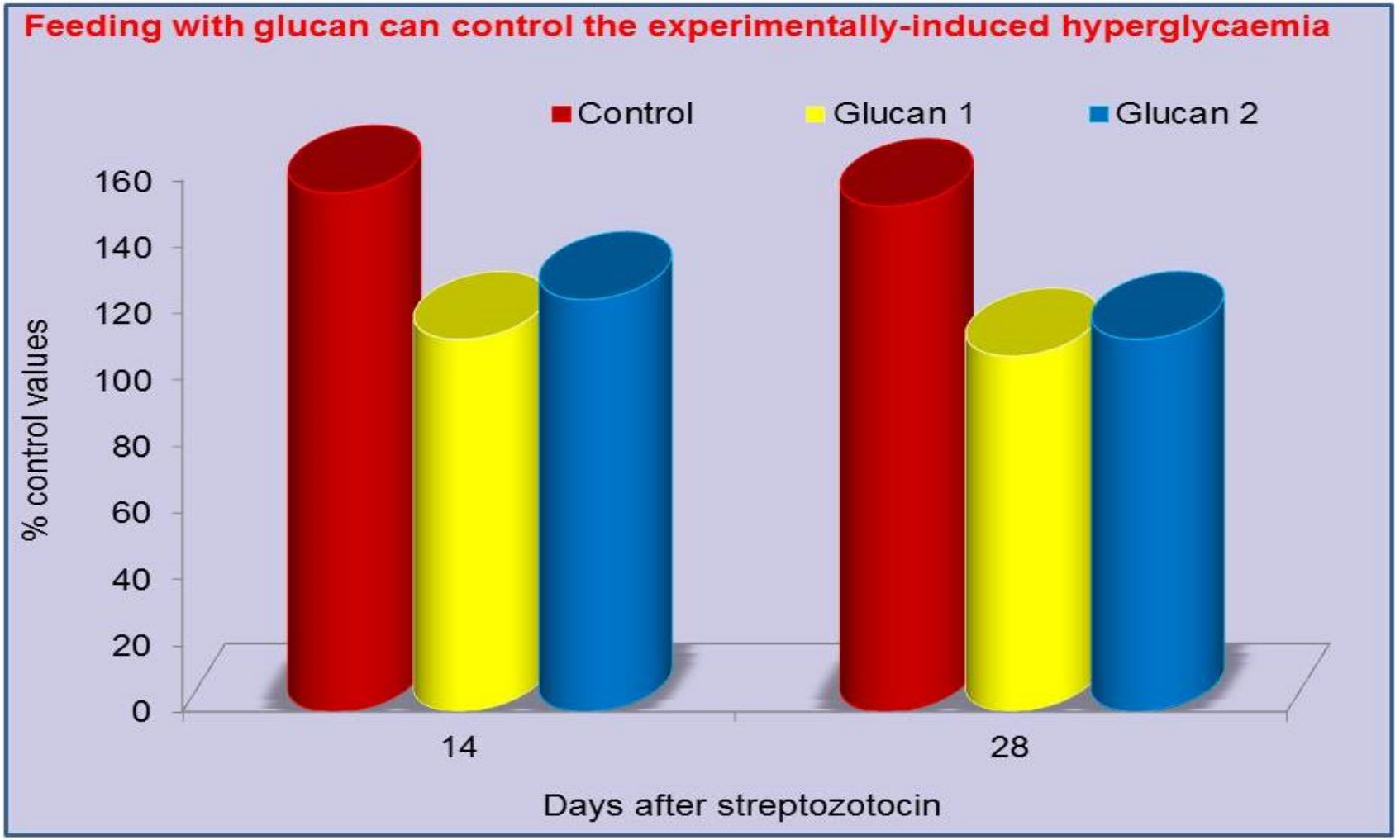
Potential in cancer treatment

- Stimulates immune cells to directly kill tumors (via CR3 and Dectin-1)
- Some glucans directly kills cancer cells (despite the fact there are no glucan receptors present)
- Activates genes involved in cancer progression
- Protects bone marrow against negative effects of chemotherapy and irradiation

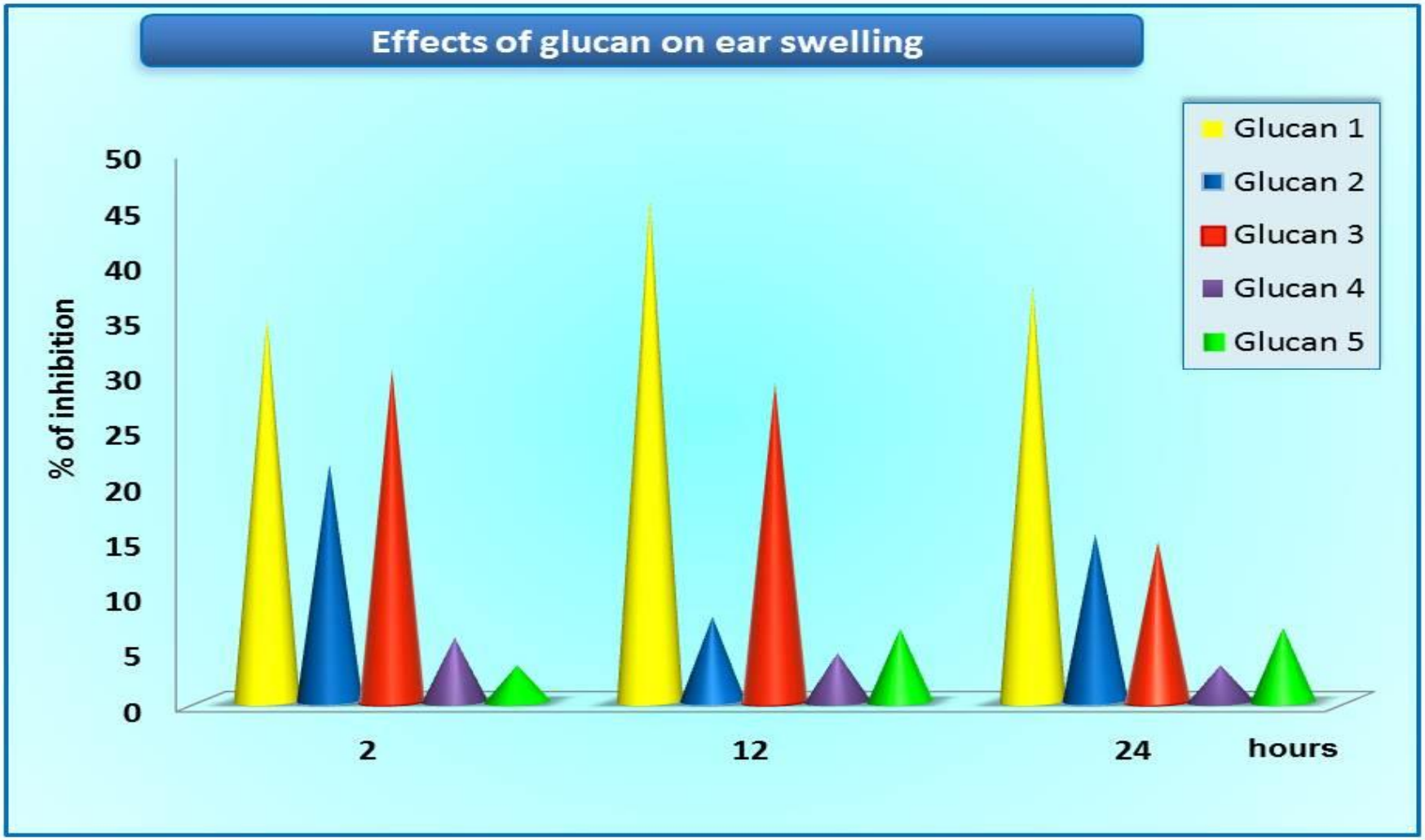
Glucan and cholesterol



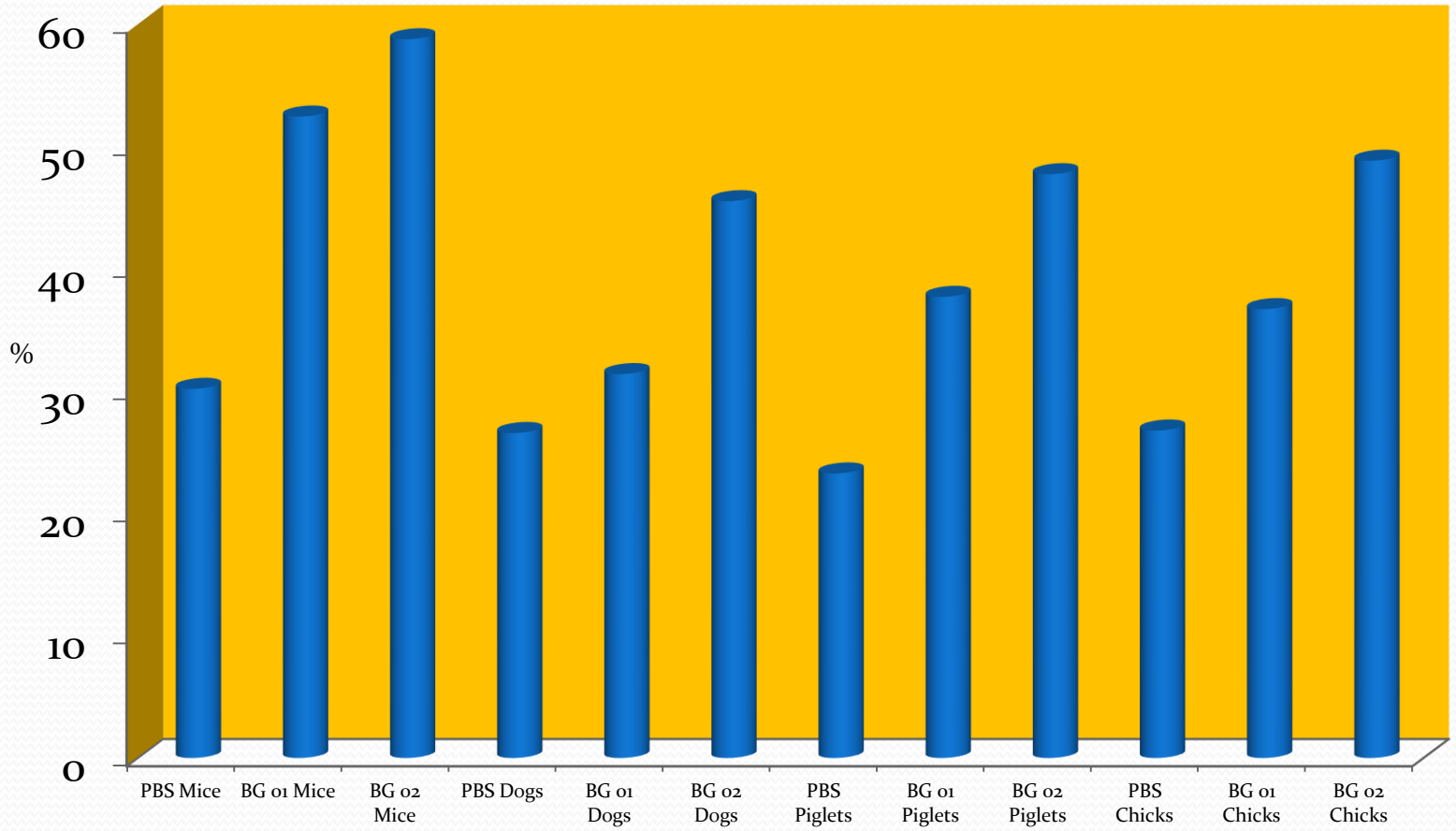
Glucan and blood sugar



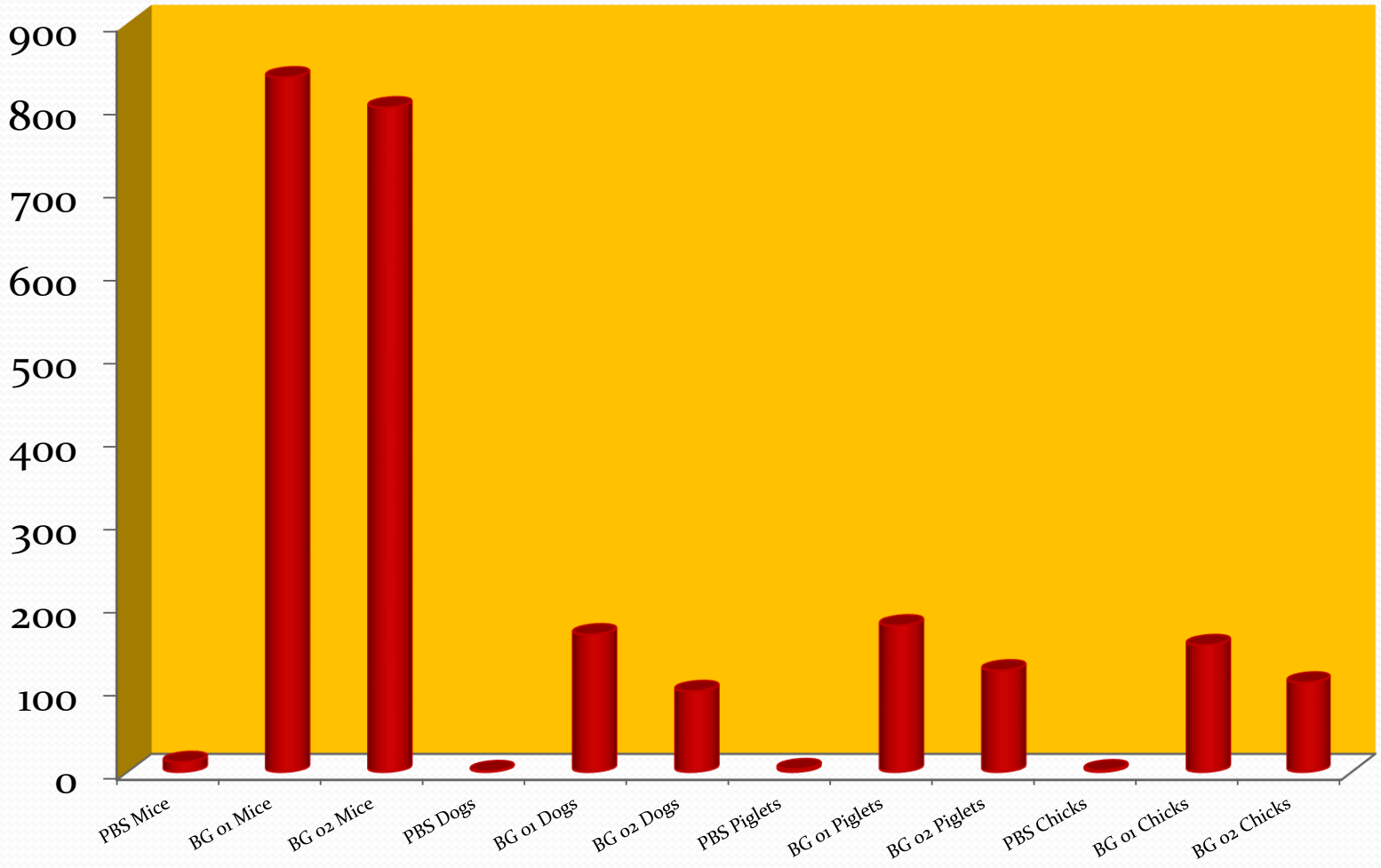
Effects on inflammation



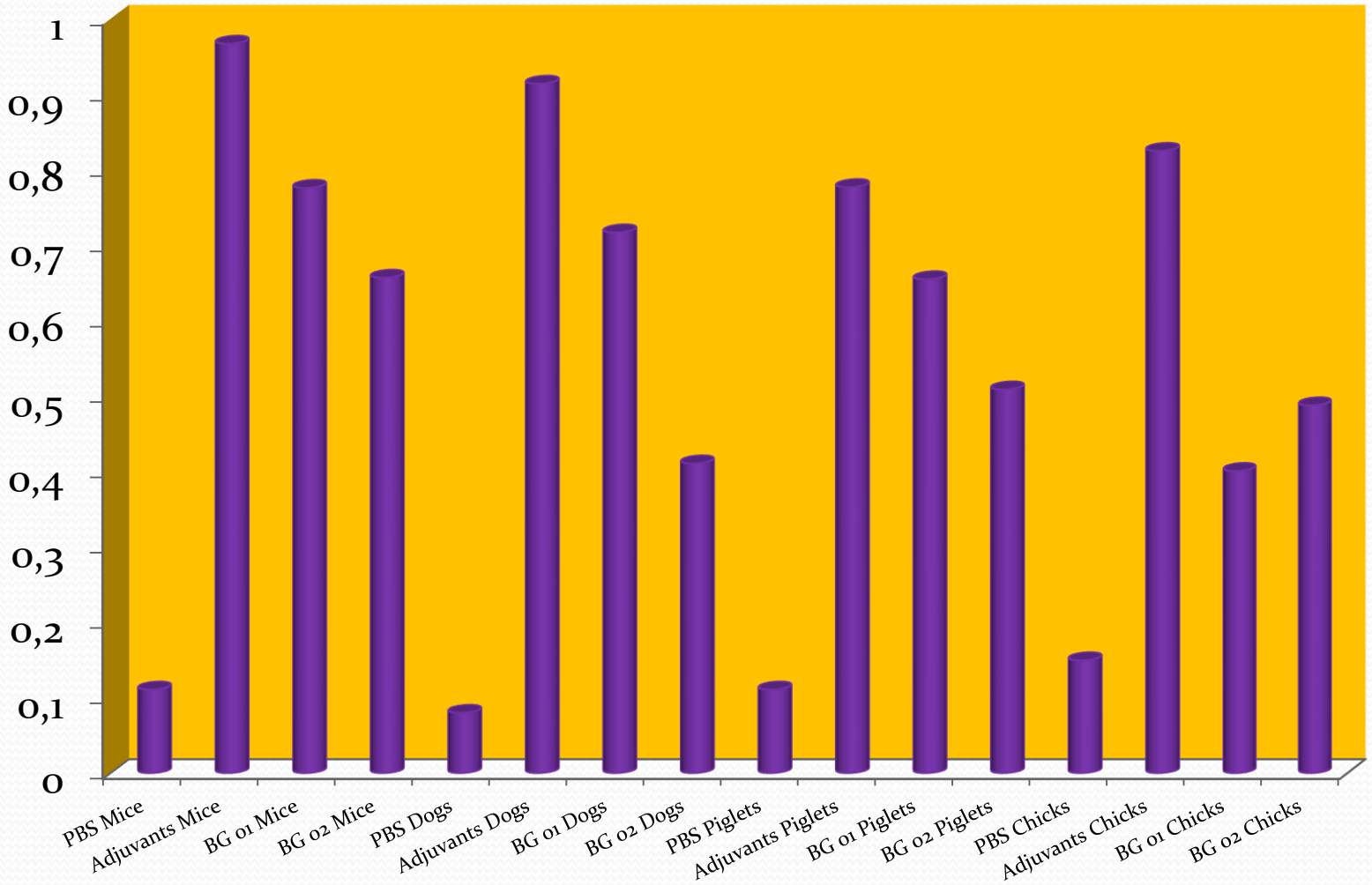
Phagocytosis (blood neutrophils)



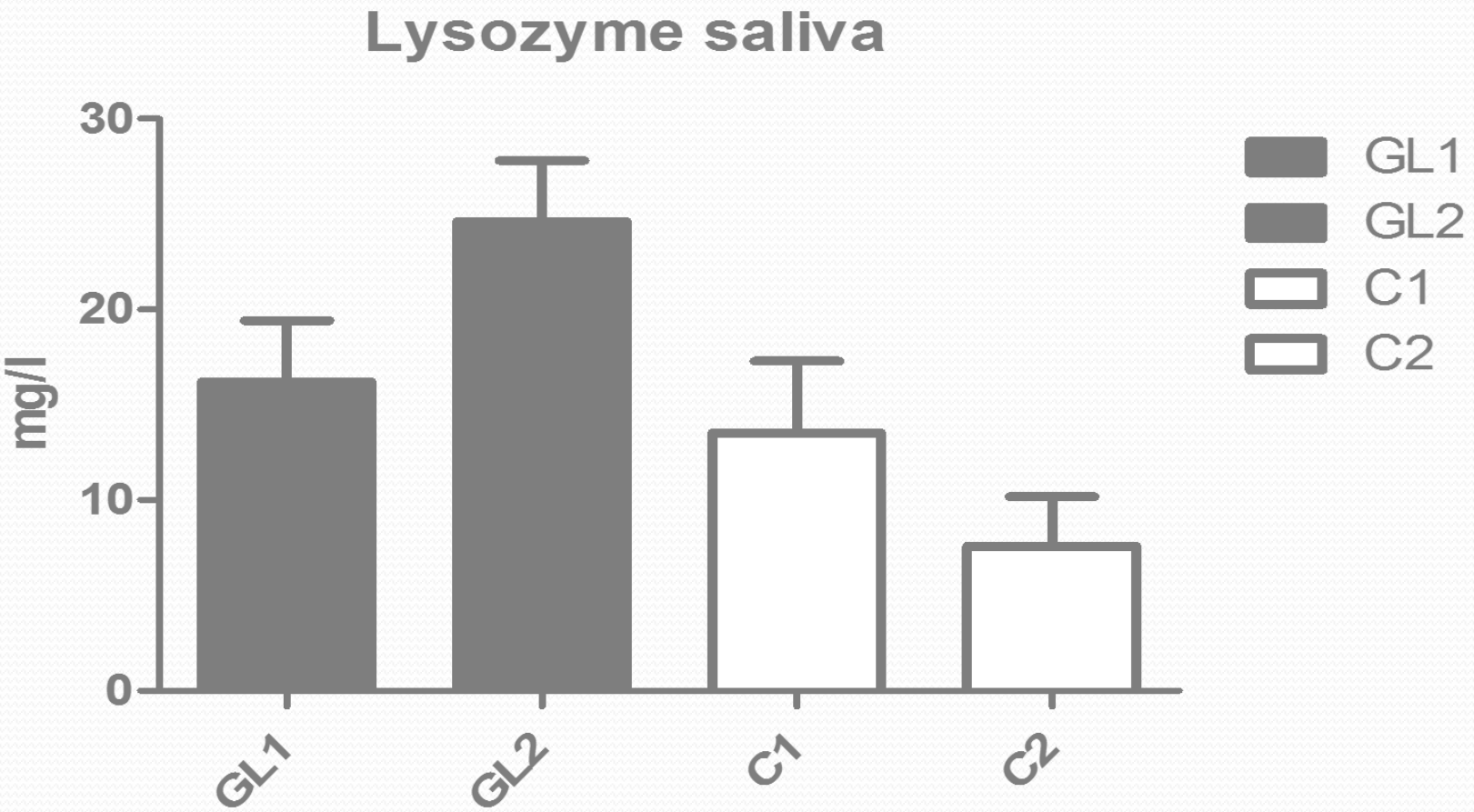
Formation of IL-2



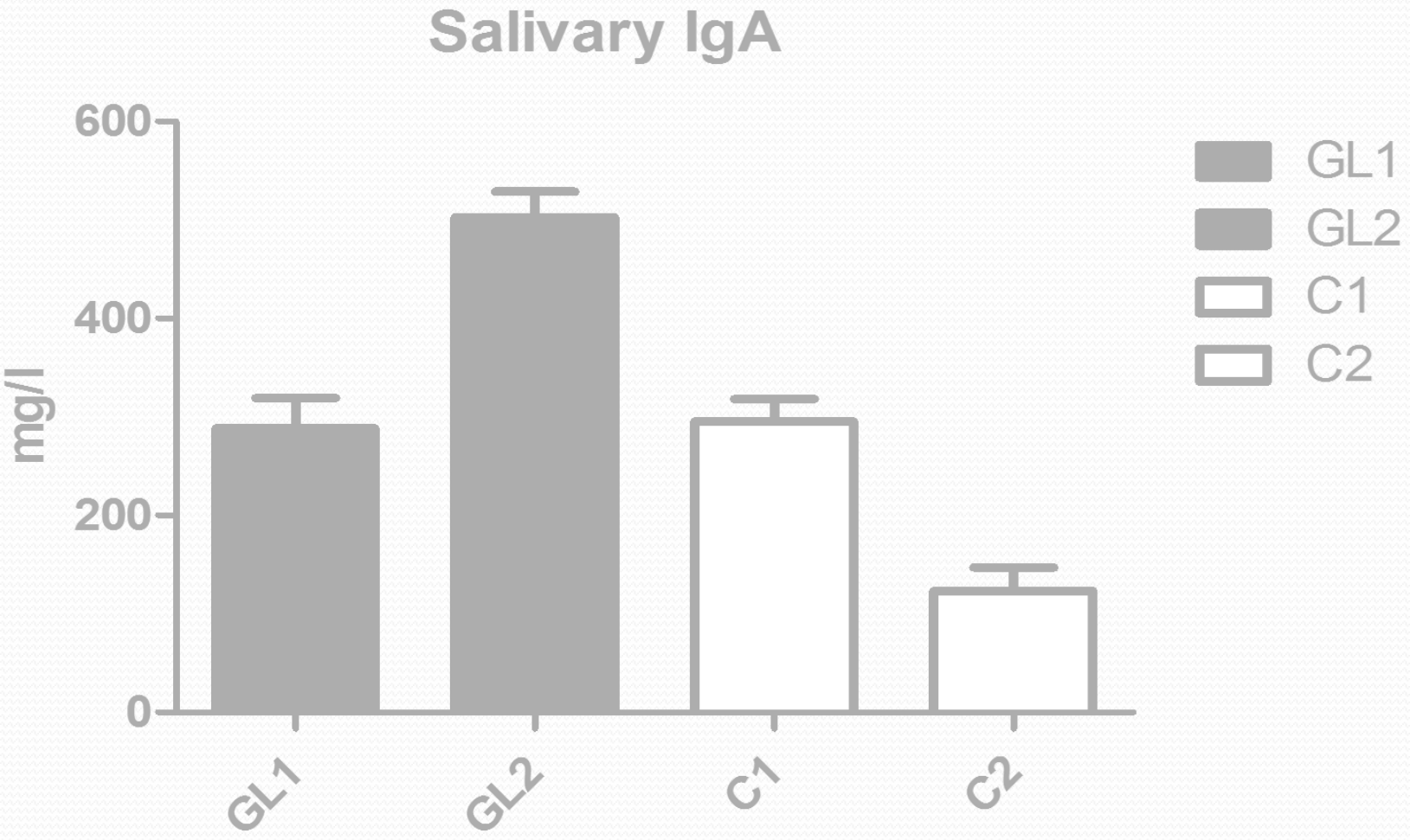
Production of antibodies



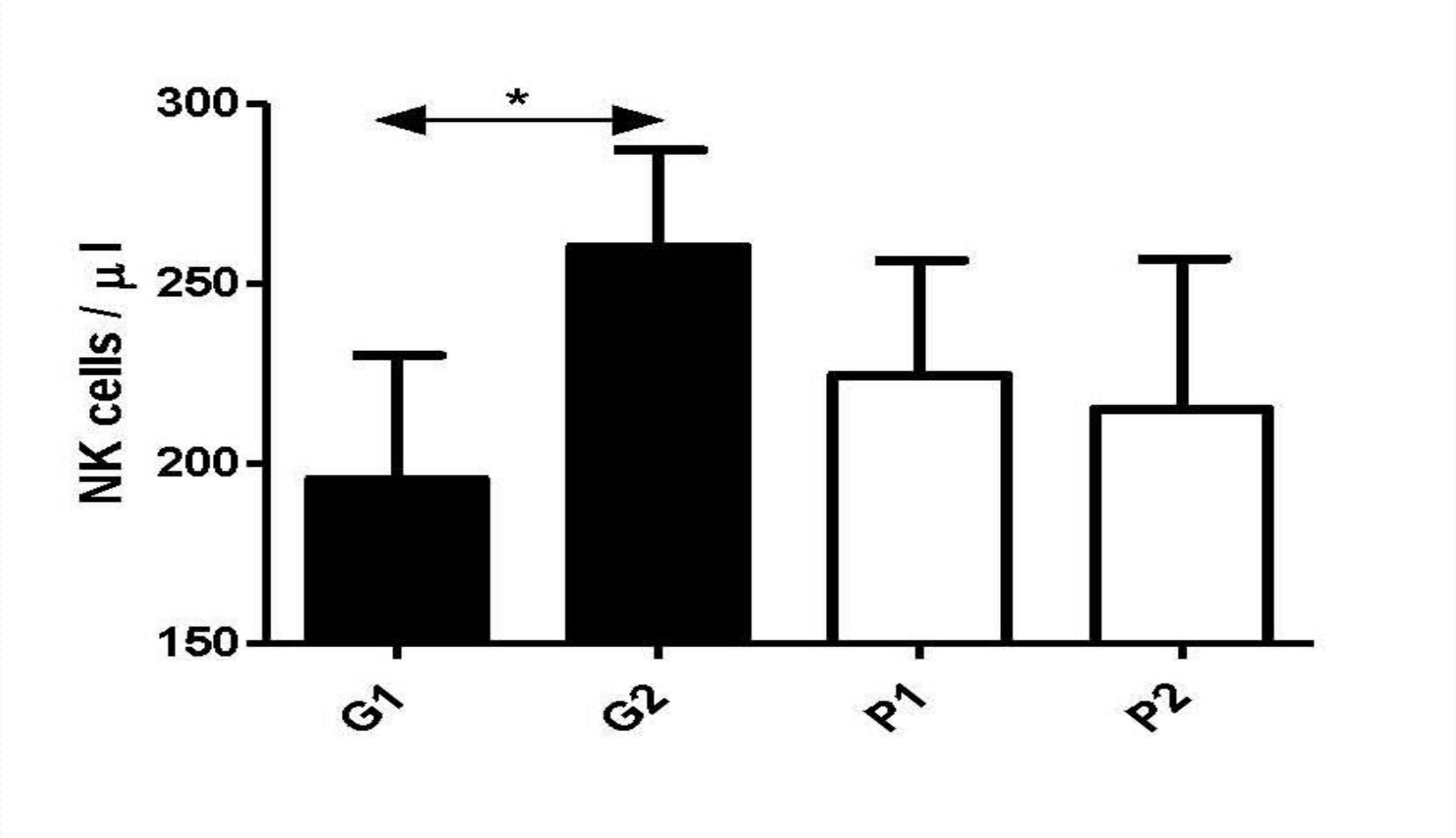
Effects of glucan on lysozyme in children



Effect of glucan on salivary IgA



Changes in NK cell activity in cancer patients



Conclusions

- Glucan is the best studied and most promising imunomodulator
- Over 15,000 published studies
- At least 69 clinical trials are currently running
- Best anti-cancer activity when used simultaneously with anti-cancer antibodies
- Works in every animal species tested

Conclusions (cont.)

- It is 100% safe
- It is natural
- Not expensive
- It has no negative side-effects
- Can be delivered in food or water
- Can be used as part of vaccines

What else do we need to know?

- Source is important
- Relation between purity and quality is complicated – high quality glucan are always pure, but higher amount of low purity glucan will not increase its activity
- No found relation between structure and function
- Price is no indicator of quality

FDA

- Listed by the FDA in the “generally recognized as safe” category with no known contraindications, side effects, or toxicity.

Approval

- In 1997, the US Food and Drug Administration (FDA) approved the use of health claim on food product packages
- In 2006 the European Food Safety Authority gave a positive opinion on health claim for food containing glucan
- It is routinely used in salmon farming

Acknowledgment

- **University of Louisville**: Gordon Ross, Jun Yan
- **Memorial Sloan-Kettering Cancer Center:** Nai-Kong Cheung
- **University of Arizona:** Bohuslav Dvořák
- **Biothera:** Gary Ostroff
- **Institute of Microbiology:** Petr Šíma
- **Hospital Usti nad Labem:** Josef Richter

