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Kinetic Estimation of Discrepancy between rats and humans regarding the Immunological response to MGN-3/Biobran

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Abstract
The interaction of MGN-3/Biobran *In vivo* in activating immune response is quite complicated and different between the rats and human. We analyze the major routes of this interaction kinetically and a cytokine mediated model (CMM) is developed to evaluate such a crucial discrepancy between the two biological systems. Rats responded with a kinetic order 1/3, while that of human was 1/24 regarding both MGN-/Biobran and INF-γ, compared to the predicted value 1/2. As well known, a higher reaction order implicates the more complicate reaction among the reactants. Alternatively, the Monod model gave values of K_s 1.43 and 2.44 mg/kg, and those of V_{max} 32.36 and 227.27%/day respectively for rats and human. Apparently, the immunological response of rats was more complicate than human in view of MGN-3/Biobran therapy.
We conclude that the discrepancy in the immune-response between rat and human can reach 3000 folds due to the inter-species variation. We suggest that such differences should be taken into account when using mice or rats as preclinical models of human disease.

Keywords: MGN-3/Biobran; Cytokine-mediated model; NK cells; Interferon-γ; Interleukin.

1. Introduction
Cancer remains the largest cause of mortality in the world, claiming over 6 millions lives each year (Abdulaev *et al*., 2000). Anticancer drug therapy induces apoptosis in cancer cells but is mostly toxic, immune-suppressive, mutagenic, and carcinogenic (Badr El-Din *et al*., 2008). Polysaccharides increase secretion of cytokines IL-1 and IL-2, which increase both the number and function of T-cells. It also enhances the differentiation of the Natural Killer (NK) cells. Polysaccharides also activate NK cells and macrophages *In vivo* so that they could swallow some abnormal and tumor cells directly by phagocytosis. By so doing, it prevents and cures cancers.
MGN-3/Biobran is an arabinoxylan extracted from rice bran that has been treated enzymatically with Shiitake mushroom extracts (Ghoneum. 1998a). MGN is a potent biological response modifier (BMR) that stimulates several different arms of the immune system including NK cells (Ghoneum & Abedi, 2004; Ghoneum 1998b; Ghoneum & Brown, 1999; Ghoneum & Jewett, 2000), T cells (Ghoneum, 1998a), B cells (Ghoneum, 1998a) and macrophages (Ghoneum & Matsuura, 2004). Accumulating evidences have demonstrated that MGN-3 has potential anticancer bioactivity (Sobajima, 1999; Mizukami, 2002; Ghoneum, 2000, Takahara, 2000; Ghoneum & Gollapudi, 2003).
NK cells have received considerable attention because of their potential role in resistance to cancer. They contribute to the first-line defense against tumor development (Herman, 1983). The main responsibilities of NK cells are surveillance against spontaneously arising tumors, destruction of tumor cells, and restriction of tumor metastasis (Sobajima, 1999**;** Mizukami, 2002; Ghoneum, 2000; Takahara, 2000; Ghoneum & Gollapudi, 2003). NK cells can kill syngeneic, allogeneic, and even xenogeneic cancer cells (Elgert, 1996a).
MGN-3/Biobran modulated cell phagocytic function by causing a significant induction of cytokines like TNF-α, IL-6, and IL-8 (Badr El-Din *et al*., 2008; Ghoneum *et al*., 2008) and IL-12 (Elgert, 1996b), and down regulation of IL-10 (Badr El-Din *et al*., 2008). In addition, high level of IL-2 can induce NK cells to differentiate into lymphokine-activated killer cells that are more effective killers of a greater variety of targets than untreated NK cells (Elgert, 1996b). Conversely, NK cells also express the α component of IL-2 receptor complex (consisting of components designated α,β, and γ), which allows NK cells to be activated by high levels of

interleukin-2 (IL-2) and interferon-γ (INF-γ). MGN-3/Biobran significantly increased INF-γ production of human peripheral blood lymphocytes (PBL) (Ghoneum *et al*., 1996a, 1999b, 2000).

The most important weapons held by NK cells in attacking cancer cells are the inherent immunoresponsive modulatory effect of MGN-3/Biobran and the expression of INF-γ, which has proven to be a potent NK modulator (Ghoneum *et al*., 1996; Ghoneum, 1996a; b; 1998a). Nonetheless, the immunological response may differ from species to species (Ghoneum *et al*., 1996a; 1999b). Two species of animals are usually used in medicinal studies, rats for animal modeling in the preclinical study, and human volunteers for the phase 0 to phase III clinical trials. The question arises to what an extent will be the interspecies variation occur? Based on the experimental data already known, we are the first who tried to use the kinetic analysis to evaluate the extent of the interspecies variation induced by MGN-3/Biobran.

2. Materials and methods
2. Material and methods
2.1. The diagrammatic model

The diagrammatic model is shown in Fig. 1, which is attached in the Supplement.

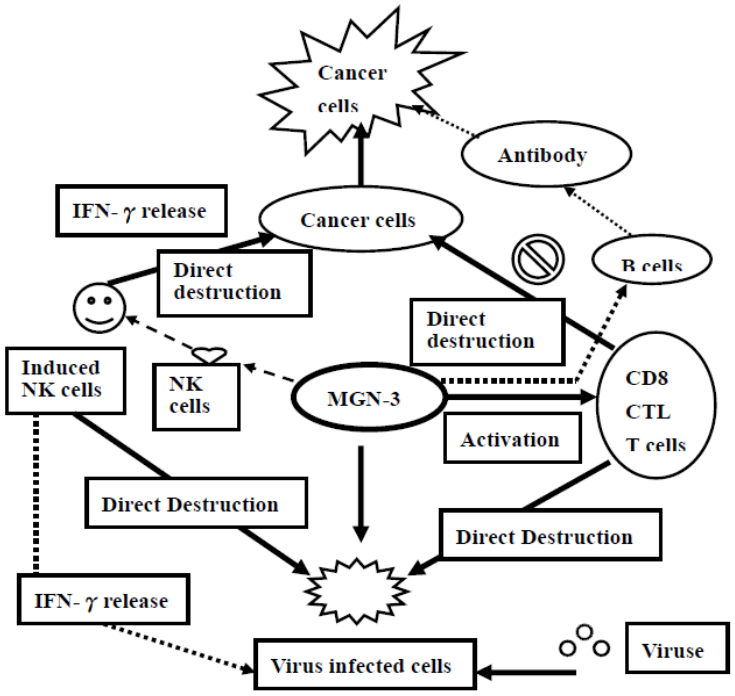

Fig 1: The Induction of NK- And Other Immune-cells By Action of MGN-3 (modified mainly by following Ghoneum *et al*., 2004)

MGN-3/Biobran induces NK cells and B cells. The induced natural killer (NK) cells are able to express INF-γ or alternatively triggers the B cells, producing antibodies to directly kill the cancer or virus-infected cells. Moreover, MGN-3 activates CD8, CTL and T cells that are also able to directly kill the cancer cells.

Table 1: INF-γ production by peripheral blood lymphocytes (PBLs) (*)

Item	Dose of MGN-3, (mg/kg)					
	0.00	**1.0**	**10.0**	**100.0**	**500.0**	**1000.0**
INF--γ production, (mg/kg)†	0.020	0.075	0.070	0.120	(0.224)	0.355
NK cytotoxicity (LU 40%/10^7 cells)‡	20.0	---	---	---	45.0	---
%	100	---	---	---	225	

*1. Data reconstructed from Ghoneum and Jewett (2000).
†2. PBLs were incubated with a wide range of MGN-3/Biobran (1-1000mg/L) alone for 16 hours.
‡3. Increased cytotoxicity mediated by peripheral blood lymphocytes (PBLs) after treatment with MGN-3/Biobran *in vitro*. PBLs from five donors were incubated with MGN-3 (0.5 mg/mL) for 16 hours.
Activity examined at effector/target (E/T) ratio of 12/1 by ^{51}Cr-release assay.
LU 40%: The number of effector cells required for 40% lysis.
4. Assume 1 L = 1 kg.

2.2. The mathematical model

The derivation of mathematical model is attached in the Supplement.

2.3. Source of data for parameter derivation

The literature sources for data collection and parameter derivation included i) Ghoneum and Jewett (2000), ii) Ghoneum (1996b) Web site: http//www.jafra.gr.jp/rat-nk-e.htm and Ghoneum (1998a).

3. Results

3.1. Parameter Reconstruction

The cited data were calculated and reconstructed as shown in Table 1-4. Table 1 shows the INF-γ production by peripheral blood lymphocytes (PBLs) (Ghoneum and Jewett, 2000); Table 2 exhibits the *In vivo* effect of MGN-3/Biobran on the activity of rat NK cells (Ghoneum, 1996b); Table 3 indicates the enhancement of human natural killer cell activity by modified arabinoxylan from rice bran (MGN-3)/Biobran (Ghoneum, 1998a), and Table 4 shows the comparison of the K_s and V_{max} values between rat and human NK Cells to MGN-3/Biobran.

3.2. Values of k' in The CM Model

By intra-polation, we obtained INF-γ production 0.224 mg/kg for dose of 500mg/kg from Table 1, substitution of values C_b = 500, [INF-γ] = 0.224, dN*/dt = 337.5%/D, we had k' = 31.9, thus Eq. 21 (see Appendix) is transformed into the general form

$$\{d[N^*]/dt\} = 31.9\{[C_b]\,[\text{INF-}\gamma]\}^{\alpha} \quad \text{----------24}$$

Eq. 24 is called herein as The Cytokine-Mediated Model (CM Model)**.**

3.3. Estimation of Monod kinetic parameters

Figures 2 and 3 were obtained from Table 2 and Table 3, from which the Linweaver-Burk plot yielded Fig. 4 and 5. On simulation of the CM Model with the values for rat in Table 2 and the values in Table 3 for human, the value of the exponential α was determined as 1/3 power for rats regarding the concentration of MGN-3 and the INF-γ produced, a result closely consistent with the predicted value 1/2 in Eq. 21. As a contrast, human showed a power only 1/24, which was far less than that of rats. More apparently, the species-dependent susceptibility to the MGN-3 was shown to be species-dependent was seen in the Linweaver Burk plots (Fig. 4 and 5). Values of K_s (mg/kg) and V_{max} (%/D) estimated to be 1.43 mg/kg and 32.36%/D; and 2.44 mg/kg and 227.27%/D; respectively for rat and human NK cells.

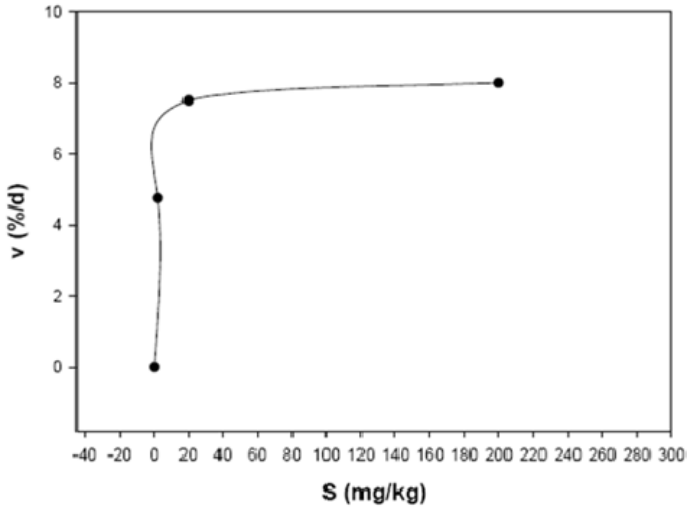

Fig 2: The proliferation rate vs. substrate plot of rat immune data.
Figure plotting adopting the reconstructed data from Ghoneum (1996b) in Table 2 of this paper.

Table 2: The *In vivo* effect of MGN-3 on the activity of rat NK cells*

Group	1	2	3	4	Remark
Dosage of MGN-3, (mg/kg/D)	0.5	5.0	50.0	Control (0.00)	
NK cell activity, (%)	119(a)	130(a)	132(a); 142 (b)	100	During a 4-day period post treatment; expressed as % of the control
Total MGN-3 administered, (mg/kg), (C_b)	2.0	20.0	200.0	0.0	During a 4-day period post treatment, herein denoted as C_b
Overall increment of NK cell activity, (%)	19	30	32	0.0	During a 4-day period post treatment
NK cell activity increment rate, (%/D), (υ)	4.75	7.50	8.00	0.0	During a 4-day period post treatment, herein denoted as υ
1/υ, (D/%)	0.211	0.133	0.125		
1/ C_b , (kg/mg)	0.5	0.05	0.005		

*Data reconstructed from Ghoneum (1996b). *In vivo* effect of MGN-3/Biobran on rat NK cell activity. Japan Functional Food Research Association (JAFRA) information.
Web site: http://www.jafra.gr.jp/rat-nk-e.htm
(a) 4 days post treatment
(b) 2 weeks post treatment 2.

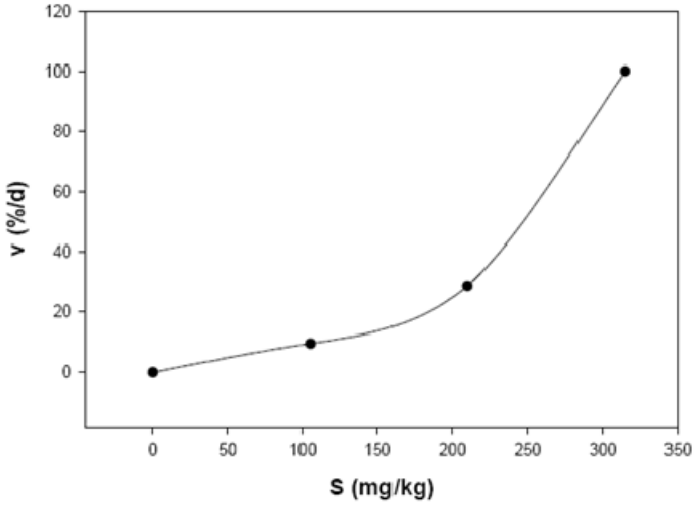

Fig 3: The proliferation rate vs. substrate plot of human immune data.
Figure plotting adopting the reconstructed data from Ghoneum (1998a) in Table 3 of this paper.

Table 3: Enhancement of human natural killer cell activity by modified arabinoxylan from rice bran (MGN-3)/Biobran*

Item	MGN-3 administered, (mg/kg/day)			Control (0.00)	Remark
	15	30	45		
NK cell activity, (%)	200% after 1 month	300% after 1 week; 500% (peak) at month 5	800% after 1 week; peak (2700%) at month 2.	Baseline: 14 lytic units = 100%	% of the control
Total MGN-3 administered, (mg/kg), (C_b)	105.0	210.0	315.0	0.0	During a 7-day period (denoted as C_b)
Net increment of NK cell activity, (%)	25.0 (a)	200.0	700.0	0.0	During 1 week (7 days) period
NK cell activity increment rate, (%/day), (υ)	3.5714; 9.595 (by extrapolation)[b]	28.57	100.00	0.0	Herein denoted as υ
1/ C_b, (kg/mg)	0.009524	0.0048	0.0032		
1/υ, (day/%)	0.2800; 0.1042 (by extrapolation) [b]	0.0350	0.0100		

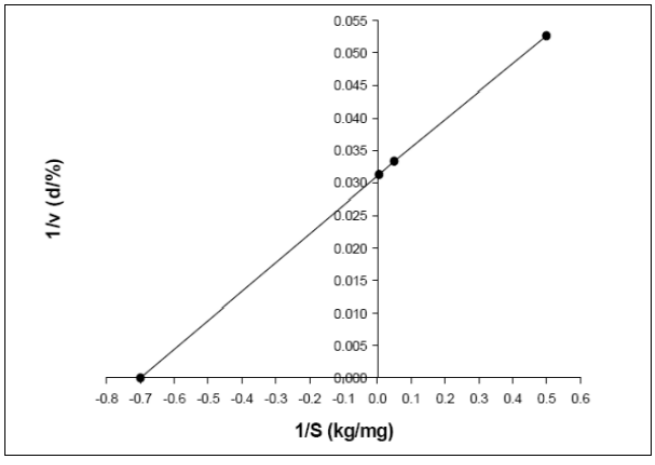

Fig 4: The Linweaver-Burk plot of rat immune data.
Figure plotting adopting the reconstructed data from Ghoneum (1996b) in Table 2 and Fig. 2 of this paper.

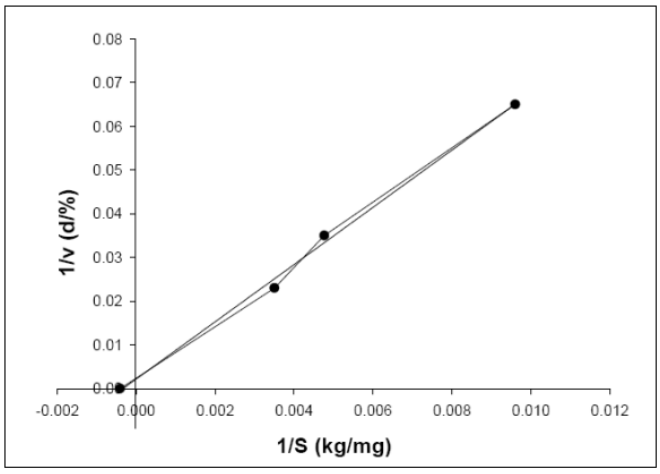

Fig 5: The Linweaver-Burk plot of human immune data
Figure plotting adopting the reconstructed data from Ghoneum (1998a) in Table 3 and Fig. 3 of this paper.

Table 4: Comparison of the K_s and V_{max} values between rat and human NK Cells to MGN-3

Source of NK cells	K_s (mg/kg)	V_{max} (%/D)	Remark
Rat	1.43	32.36	a 4-day response
Human	2.44	227.27	a 7-day response

4. Discussion

Similar to the pharmacological action mechanism exerted by MGN-3/Biobran, *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae) polysaccharide (GLP) also possesses antitumor and NK cell activating capability (Lin *et al*., 2005). GLP activates monocyte derived dentritic cells (DC), cell surface expression of CD80, CD86, CD83, CD40, CD54, and human leucocyte antigen (HLA)-DR, as well as the production of IL-12p70, p40 and IL-10 and also IL-12p35, p40, and IL-10 mRNA expression (Lin *et al*., 2005). The capacity for endocytosis of DC was suppressed by GLP. Treatment of DC with GLP resulted in increased T cell-stimulating capacity and T-cell secretion of INF-γ and IL-10 (Lin *et al*., 2005). Thus, the action mechanism of polysaccharides on immunoresponse can be very complicate, depending on the source, the molecular size, and the chemical structure of polysaccharides.

Moreover, GLP induced a marked increase in the expression levels of interleukin (IL)-lα (2 folds), IL-lβ (3 folds), TNF-α (2 folds), IL-12 p35 (up to 6 folds), and IL-12 p40 (3 folds) (Ooi *et al*., 2002). In macrophages, GLP promoted a remarkable increase in the expression levels of IL-β (2.5 to 3 folds), TNF-α (up to 6 folds), and macrophage colony-stimulating factor (M-CSF) (up to 2 folds) (Ooi *et al*., 2002). These results indicate that antitumor GLP is able to induce a cascade of immunomodulatory cytokines, but the potential of their gene expression and interaction seemed to be quite complicated (Ooi *et al*., 2002). Moreover, the TNF-α induction in macrophage is much more up-regulated by smaller GLP molecules (MW<12000), indicating the molecular size to be one of the determinant factors in the structure-function relationship of the polysaccharide therapy (Ooi *et al*., 2002). In parallel, clinically when the molecular size of MGN-3/Biobran was reduced from 500 kDa to 50 kDa, the overall therapeutic effect can be enhanced 2-4 folds depending on the tumor type and stages (Personal communication from Daiwa Pharmaceutical Co., Japan), results being rather consistent with Ooi *et al*. (2002).

Animal models have long been used to test theories of and therapies for chronic inflammatory diseases. The use of rodents is widespread among researchers: mice have been used for models of cancer and any number of chronic diseases. The study on their immune responses has yielded tremendous insight into the workings of the human immune system. However, in view of evolution history, a 65 million-year of evolution might have created significant interspecies differences. Recent evidences have revealed discrepancies in both innate and adaptive immunity, including: balance of leukocyte subsets, defensins, Toll receptors, inducible NO synthase, the NK inhibitory receptor families Ly49 and KIR, FcR, Ig subsets, the B cell (BLNK, Btk, and λ5) and T cell (ZAP70 and common γ-chain) signaling pathway components, Thy-1, γδT cells, cytokines and cytokine receptors, Th1/Th2 differentiation, costimulatory molecule expression and function, Ag-presenting function of endothelial cells, and chemokine and chemokine receptor expression (Mestas and Hughes, 2004). In addition, the pathogenesis of multiple sclerosis and delayed-type hypersensitivity, where complex multicomponent processes differ may exhibit much more discrepancy (Mestas and Hughes, 2004). Earlier, similar phenomenon had been raised with a strong evidence showing that the regulation of expression of *mdr* genes in liver cells in response to carcinogens such as 2-acetylaminofluorene (2-AAF) is gene- and species-specific (Lecureur *et al*., 1996). More interestingly, MDR1 and Mdr1b, but not Mdr2, are able to transport permanently charged derivatives of quinidine and quinine and both MDR1 and Mdr1b display stereospecificity in favor for the (*R*)-diastereomer *N*-methylquinidine as compared to the (*S*)-diastereomer *N*-methylquinine, yet human MDR1 is more efficient in transporting the cationic model compounds than its rat orthologue Mdr1b (Hooiveld *et al*., 2002).

Multiple studies have demonstrated that the immune systems of rats and humans are inherently different. Molecular modeling research has revealed that the activity of the human innate immune system is controlled by the Vitamin D Receptor (VDR). (http://mpkb.org/home/patients/assessing_literature/animal_models) In humans, the VDR not only controls the activity of the innate immune system, but it transcribes 913 genes, and possibly more. In contrast, the rat innate immune system is not controlled by the VDR. It depends on a cascade of nitric oxide (an important signaling molecule) that functions in a manner yet to be fully understood

(http://mpkb.org/home/patients/assessing_literature/animal_models). Rats do have VDR, but they transcribe different genes than the human VDR. By using molecular modeling software, researchers at McGill University in Canada found many differences in the genes targeted by the rat and human VDR. For example, the gene encoding a calcium binding protein called osteocalcin is "robustly" transcribed by the VDR in humans, but not in mice. In what proves to be a fundamental difference between mice and men, Brahmachary *et al*. recently determined that the rat VDR does not express the cathelicidin antimicrobial peptides – marking an important difference in the way the two species kill invading pathogens. This means that rats and humans respond differently to molecules or drugs that affect the VDR and subsequently the innate immune system. (http://mpkb.org/home/patients/assessing_literature/animal_ models).

Based on the cytokine-mediated model (CM Model, see Appendix), the kinetic order α in Eq. 24 related with rats and human was 1/3 and 1/24 respectively with respect to INF-γ expression. The overall deviation reaches 3000 folds [antilog(1/3)/(1/24) = antilog 8 = 3000] (Also be referred to Fig. 2 and Fig. 3). In general chemical kinetic analysis, a higher reaction order would implicate the more complicate the reaction to be depending on the reactants and the reaction condition. Nonetheless although rats evidently exhibited more complicate and slower immunological response than human regarding the MGN-3/Biobran therapy, such a deviation in fact may arise from the interspecies immunological discrepancy.

Suggestively, such differences should be taken into account when using mice or rats as the preclinical models to investigate the human diseases.

5. Conclusion

The discrepancy in the immunoresponse kinetic order of MGN-3/Biobran used for cancer therapy can reach 3000 folds comparing the rodents with that of humans. The discrepancy arises due to the inter-species variation. We suggest that such differences should be taken into account when using mice or rats as preclinical models of human disease.

Appendix

Mathematical Modeling

A1. Nomenclature

Notation	Unit	Description
ASC	Cell count/mL	The population of altered self cells
B	Cell count/mL	The population of B cells (plasma cells)
B*	Cell count/mL	The population of the activated B cells (plasma cells)
Ca	Cell count/mL	The population of cancer cells
C_b	mg/kg	Dosage of MGN-3 (Bio-Bran) administered
CTL	Cell count/mL	The population of cytotoxic T lymphocytes
INF-γ	mg/mL	The concentration of interferon-γ
K_s	mg/kg	The half-saturation constant
MHC class II	mg/mL	Major histocompatibility complex class II
N	Cell count/mL	The population of the natural killer cells
N*	Cell count/mL	Induced population of the natural killer cells
NK	Cell count/mL	The population of the natural killer cells
Tc	Cell count/mL	The population of cytotoxic T cells
Tc*	Cell count/mL	The population of the activated cytotoxic T cells
T_H*	Cell count/mL	The population of the activated helper T cells
T_H	Cell count/mL	The population of the helper T cells
VIC	Cell count/mL	The population of the virus infected cells
V_{max}	%/D	Percent maximum induction rate of NK cells per day
K	----	The population constant of NK cells
k'	$\%kg^{1/\alpha}/mg^{1/\alpha}$-D	The α^{th} root of K
υ	%/D	Percent induction rate of the NK cells per day
d[INF-γ]/dt	mg/kg-D	The production rate of interferon-γ per kg per day
d[N*]/dt	%/D	Percent induction rate of NK cells
k_1	kg/mg-D	The induction rate constant of NK cells by MGN-3
k_2	kg/cell-D	The induction rate constant of NK cells by INF-γ

A2. The Mathematical Model Derivation

A2.1. The Cytokine-Mediated Model (CM Model)

A2.1.1. The Diagrammatic Model

Laboratory experiments have shown that the MGN-3/Biobran-relevant immune system can be described briefly as shown in Fig. 1 (Ghoneum, 1999a; 1999b; Goldsby *et al*., 2001).

The activation process of T_H cells to form activated T_H cells (Effector cell, T_H*) is mediated by the innate major histocompatibility complex (MHC) class II (Eq. 1), which in turn induces B cells, Tc cells, and NK cells to produce induced B* cells (Eq. 2), induced CTL (Tc* **)** (Eq. 3), and induced NK cells (N*) (Eq. 4), respectively. Eqs. 2-4 are strongly modulated by MGN-3/Biobran, especially Eq. 4 (Ghoneum, 1999a;1999b).

T_H cell $\xrightarrow{\text{MHC class II}}$ Activated T_H cell (effector cell, T_H^*) ------ 1

B cell $\xrightarrow{\text{MGN-3, } T_H^*}$ Induced B cell, plasma cell (B^*) ---------- 2

T_H cell $\xrightarrow{\text{MGN-3, } T_H^*}$ Induced CTL (T_c^*) ---------- 3

NK cell $\xrightarrow{\text{MGN-3, } T_H^*}$ Induced NK cell (N*) ---------- 4

$N^* + CA \longrightarrow$ Dead CA cells ---------- 5

$CA + N^* \longrightarrow$ Dead N by CA phagocytosis ---------- 6

Induced NK cells (N*) destroy the cancer cells (CA) (Eq. 5) or the vice versa (Eq. 6)

Alternatively, the virus infected cells (VIC) or the altered self-cells (ASC) are directly attacked by the induced NK cells (Eq. 7) or the induced Tc* (Eq. 8):

VIC or ASC + N* $\rightarrow$ Destroyed VIC or ASC ----------7

VIC or ASC + Tc* $\rightarrow$ Destroyed VIC or ASC ----------8

In addition, the induced NK releases INF-γ, which in turn activates the NK cells. Thus, the diagrammatic model (Fig. A1) is simplified to the induction of NK cells (N*) when treated with MGN-3/Biobran (C_b) (Eq. 9), and the auto-catalytic activation of NK cells by INF-γ (Eq. 10) to give

$N + C_b \rightarrow N^* + INF\text{-}\gamma$ ----------9

$N + INF\text{-}\gamma \rightarrow N^*$ ----------10

A2.2. The Mathematical Model

The induction of NK cells (N*) by MGN-3/Biobran and the subsequent auto-catalytic production of INF-γ are shown as Eq. 9 and Eq. 10.

From which the rate for induction of NK cells is

$d[N^*]/dt = d[INF\text{-}\gamma]/dt = k_1 [N] [C_b]$ ----------11

On rearrangement of Eq. 9 we have

$k_1 = \{d[N^*]/dt\}/ [N] [C_b]$ ----------12

and

$k_1 = \{d[INF\text{-}\gamma]/dt\}/ [N] [C_b]$ ----------13

and from Eq. 10 we have

$d[N^*]/dt = k_2 [N] [INF\text{-}\gamma]$----------14

or

$k_2 = \{d[N^*]/dt\}/[N] [INF\text{-}\gamma]$ ----------15

Multiplication of Eqs.12 and 15 yields the overall rate constant

$k = \{d[N^*]/dt\}^2/[N]^2 [C_b] [INF\text{-}\gamma]$ ----------16

where $k = k_1k_2$ ----------17

Eq. 16 states that the overall rate constant k for the induction of NK cells can be calculated from Eq. 16, provided the parameters involving the increment rate of induced NK cells, d[N*]/dt; the original total NK cells population; N, the concentration of INF-γ, [INF-γ]; and the dosage of MGN-3/Biobran (C_b) are available.

Rearrangement of Eq. 16 yields

$\{d[N^*]/dt\}^2 = k [N]^2 [C_b] [INF\text{-}\gamma]$ ----------18

Normally, the total NK cell population N is considered to be a constant in each individual within a relatively short initial transit period, in which MGN-3/Biobran is responsible for the induction of only a small fraction of total NK cells without affecting the total population of NK cells (Ghoneum, 1998a; 1998b). Eq. 18 is further simplified as

$\{d[N^*]/dt\}^2 = K [C_b] [INF\text{-}\gamma]$ ----------19

Here $K = k [N]^2$

Squared root of Eq. 19 gives

$\{d[N^*]/dt\} = k'\{[C_b] [INF\text{-}\gamma]\}^{1/2}$ ----------20

Or in general form

$\{d[N^*]/dt\} = k'\{[C_b] [INF\text{-}\gamma]\}^{\alpha}$ ----------21

Where, $k' = \sqrt{K}$, which states that the induction rate of NK cells (d[N*]/dt) is directly proportional to the squared root of the product of INF-γ and the MGN-3/Biobran (C_b) concentration. Apparently, the result yields a non-linear relationship between the treatment efficiency and the dosage of MGN-3/Biobran. Herein Eq. 21 is named The Cytokine-Mediated Model (CM Model).

Alternatively, assume Monod kinetics to be applicable to the immune response (note: the Monod kinetics in fact is applicable to cell proliferation), we have the equation

$\mu = \mu_{max} \bullet C_b/K_s + C_b$ ----------22

In reality, the cell proliferation rate is directly proportional to the specific growth rate, hence

$\upsilon = \mu N$, and

$V_{max} \cdot = \mu_{max} N$

Thus

$\upsilon = V_{max} \cdot C_b /K_s + C_b$ ----------23

Where, υ is the production rate of induced NK cells, dN*/dt; V_{max} is the maximum production rate of induced NK cells; C_b is the dosage of MGN-3/Biobran administered (for practical use, see Eq. 24 in the text).

6. Acknowledgement

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7.Conflict of Interest and Disclosure Statement

The authors have disclosed any conflict of interest.

8. References

1. Abdullaev FI, Rivera-Luna R, Roitenburd-Belacortu V, Epsinosa-Aguirre J. Pattern of childhood cancer mortality in Mexico. Arch Med Res 2000; 31(5):526-31.
2. Badr El-Din NK, Noaman E, Gohneum M. *In vivo* tumor inhibitory effects of nutritional rice bran supplement MGN-3/Biobran on Ehrlich carcinoma-bearing mice. Nutrition and Cancer, 2008; 60(2):235-44.
3. Elgert KD(ed). IMMUNOLOGY, Wiley-Liss, Inc., 605 Third Avenue, New York, NY 10158-0012, USA. 1996a; 23-4.
4. Elgert KD(ed). IMMUNOLOGY, Wiley-Liss, Inc., 605 Third Avenue, New York, NY 10158-0012, USA.1996b; 32.
5. Ghoneum MH. Anti-HIV activity By MGN-3 *in vitro*. 9th International Conference on AIDS, July 7-12, 1996a, Vancouver, BC.
6. Ghoneum MH. *In vivo* effect of MGN-3 on rat NK cell activity. Japanese Functional Food Research Association. 1996b.
7. Web site: http//www.jafra.gr.jp/rat-nk-e.htm
8. Ghoneum MH, Namatalla G, Kim C. Effect of MGN-3 on human natural killer cell activity and interferon-γ synthesis *in vitro*. FASEB JOURNAL, New Orleans, LA, 1996. FASEB 1996; 10:26-32.
9. Ghoneum M. Anti-HIV activity *in vitro* of MGN-3, an activated arabioxylan from rice bran. Biochem Biophys Res Commun 1998; 243(1):25-9.
10. Ghoneum M. Enhancement of human natural killer activity by modified arabinoxylan from rice bran (MGN-3). Int J Immunother 1998b; 14:89-99.
11. Ghoneum M, Brown JNK. Immunorestoration of cancer patients by MGN-3, a modified arabinoxylan rice bran (study of 32 patients followed for up to 4 years). In anti-aging Medical therapeutics, volume 111, Klatz R, Goldman R (eds). Marina del Rey, CA: Health Quest Publications, 1999; 217-26.
12. Ghoneum M, Jewett A. Production of tumor necrosis factor–alpha and interferon-gamma from human peripheral blood lymphocytes by MGN-3 *in vitro*. Cancer Detect Prev 2000; 24(4):314-24.
13. Ghoneum MS, Gollapudi S. MGN-3 potentiates death receptor-induced apoptosis in cancer cells: paper presented at the 93rd Annual Meeting 2003 of American Association for Cancer Research, Boston, USA by M. Ghoneum and S. Gollapudi, Drew University of Medicine and Science, Los Angeles, CA, UC Irvine, Division of

Basic and Clinical Immunology, Irvine CA, 2003.
14. Ghoneum MH. Enhancement of human natural killer cell activity by modified arabinoxylan from rice bran (MGN-3). Int J Immunother 1998a; 14:89-99.
15. Ghoneum M. Anti-HIV activity *in vitro* of MGN-3, An activated Arabinoxylan From Rice Bran. Biochem Biophysi Res Communi 1998b; 243:25-9.
16. Ghoneum MH. Immunostimulation and Cancer Prevention. Anti-Aging Conference & Exposition, 7th International Congress on Anti-Aging & Biomedical Technologies, December 11-13,1999. Tropicana Hotel. Las Vegas, NV, Pre-conference 1999a; 10-2.
17. Ghoneum MH, Brown JNK. Immunorestoration of Cancer Patients by MGN-3, A Modified Arabinoxylan Rice Bran (Study of 32 Patients Followed for up to 4 Years). Anti-aging Med Ther 1999b; 3:217-26.
18. Ghoneum MH. (ed) Anticancer Power---Arabinoxylan MGN-3; Japanese Version, 1998, Chinese Translation, Edn 1, 1999c, 118-22.
19. Ghoneum MH, Jewett A. Production of tumor necrosis factor-α and interferon-γ from human peripheral blood lymphocytes by MGN-3, modified arabinoxylan from rice bran, and its synergy with interleukin-2 *in vitro*. Cancer Det Prev 2000; 24(4):314-24.
20. Ghoneum M., Matsuura M. Augmentation of macrophage phagocytosis by modified arabinoxylan rice bran (MGN/Biobran). Int J Immunopath Pharmacol 2004; 17(3):283-92.
21. Ghoneum M, Abedi S. Enhancement of natural killer cell activity or aged mice by modified arabinoxylan rice bran (MGN-3/Biobran). J Pharm Pharmacol 2004; 56(12): 1581-8.
22. Ghoneum M, Matsuura M, Gollapudi S. Modified arabinoxylan rice (MGN-3/Biobran) enhances intracellular killing microbes by human phagocytic cells *in vitro*. Int J Immunopath Pharmacol 2008; 21(1):87-95.
23. Goldsby RA, Kindt TJ, Osborne BA. (eds). Kuby Immunology, Edn 4, 2000. W. H. Freeman and Company, New York, USA, 2001, 12-3.
24. Herman RB. Natural killer cells and tumor immunity. Surv Immun Res 1983; 2:306-8.
25. Hooiveld GJEJ, Heegsma J, van Montfoort JE, Jansen PJM, Meijer DKF, Müller M *et al*. Stereoselective transport of hydrophilic quaternary drugs by human MDR1 and rat Mdr1b P-glycoproteins. Br J Pharmacol 2002; 135(7):1685-94.
26. Lin YL, Liang YC, Lee SS, Chiang BL. Polysaccharide purified from Ganoderma lucidum induced activation and maturation of human monocyte-derived dendritic cells by the NF-kappaB and p38 mitogen-activated protein kinase pathways. J Leuko Biol 2005; 78(2):533-43.
27. Lecureur V, Guillouzo1 A, Fardel O. Differential regulation of mdr genes in response to 2-acetylaminofluorene treatment in cultured rat and human Hepatocytes. Carcinogenesis 1996; 17(5):1157-60.
28. Mestas J, Hughes CCW. Of Mice and Not Men: Differences between Mouse and Human Immunology. J Immunol 2004; 172(5):2731-8.
29. Mizukami O. (Health Promotion Research Institute New Life Layman Foundation, JAPAN): Abstract from Biobran Workshop in Berlin. A case in which Biobran MGN-3 was used as a supplemental treatment when curing metastasis from lung cancer, 2002.
30. Ooi LSM, Ooi VEC, Fung MC. Induction of Gene Expression of Immunomodulatory Cytokines in the Mouse by a Polysaccharide from Ganoderma lucidum (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). Int J Med Mushrooms 2002; 4:80.
31. Sobajima T. (Hoshigaoka Kosei Nenkin Hospital, JAPAN): The Abstract of the 2nd Annual Meeting of the Japanese Society for Complementary & Alternative Medicine & Treatment, Effectiveness of Biobran MGN-3 against Tumor cell Growth, 1999.
32. Takahara K. (Sano Surgical Clinic, JAPAN) The Abstract of the 3rd Annual Meeting of the Japanese Society for Complementary & Alternative Medicine & treatment, 2000.