Yeh Chen, Hui-Er Wang, Jing-Gung Chung, Robert Y. Peng

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-\(\gamma\), 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-\(\gamma\); 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 arabinobxylan 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-\(\alpha\), 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 \(\alpha\) component of IL-2 receptor complex (consisting of components designated \(\alpha, \beta, \) and \(\gamma\)), 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>
<thead>
<tr>
<th>Item</th>
<th>Dose of MGN-3, (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>INF-γ production, (mg/kg)¹</td>
<td>0.020</td>
</tr>
<tr>
<td>NK cytotoxicity (LU 40%/10⁷ cells)²</td>
<td>20.0</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
</tr>
</tbody>
</table>

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 ⁵¹Cr-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

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

\[ \frac{d[N^*]}{dt} = 31.9 \cdot [C_b] \cdot [INF-\gamma]^\alpha \]

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 Eq. 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 $\alpha$ 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.](image)

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

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


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* 

<table>
<thead>
<tr>
<th>Item</th>
<th>MGN-3 administered, (mg/kg/day)</th>
<th>Control (0.00)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>NK cell activity, (%)</td>
<td>200% after 1 month</td>
<td>300% after 1 week; 500% (peak) at month 5</td>
<td>Baseline: 14 lytic units = 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>800% after 1 week; peak (2700%) at month 2</td>
<td>% of the control</td>
</tr>
<tr>
<td>Total MGN-3 administered, (mg/kg), (Cₚ)</td>
<td>105.0</td>
<td>210.0</td>
<td>315.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td>During a 7-day period (denoted as Cₚ)</td>
</tr>
<tr>
<td>Net increment of NK cell activity, (%)</td>
<td>25.0 (a)</td>
<td>200.0</td>
<td>700.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td>During 1 week (7 days) period</td>
</tr>
<tr>
<td>NK cell activity increment rate, (%/day), (υ)</td>
<td>3.5714; 9.595 (by extrapolation)</td>
<td>28.57</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td>Herein denoted as υ</td>
</tr>
<tr>
<td>1/Cₚ, (kg/mg)</td>
<td>0.009524</td>
<td>0.0048</td>
<td>0.0032</td>
</tr>
<tr>
<td>1/υ, (day/%)</td>
<td>0.2800; 0.1042 (by extrapolation)</td>
<td>0.0350</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

Fig 4: The Lineweaver-Burk plot of rat immune data.
Figure plotting adopting the reconstructed data from Ghoneum (1996b) in Table 2 and Fig. 2 of this paper.
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, Bik, and l,5) 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.
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. 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 complicated the reaction to be depending on the reactants and the reaction condition. Nonetheless although rats evidently exhibited more complicated 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.

### Appendix

#### Mathematical Modeling

<table>
<thead>
<tr>
<th>Notation</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC</td>
<td>Cell count/mL</td>
<td>The population of altered self cells</td>
</tr>
<tr>
<td>B</td>
<td>Cell count/mL</td>
<td>The population of B cells (plasma cells)</td>
</tr>
<tr>
<td>B*</td>
<td>Cell count/mL</td>
<td>The population of the activated B cells (plasma cells)</td>
</tr>
<tr>
<td>Ca</td>
<td>Cell count/mL</td>
<td>The population of cancer cells</td>
</tr>
<tr>
<td>Cs</td>
<td>mg/kg</td>
<td>Dosage of MGN-3 (Bio-Bran) administered</td>
</tr>
<tr>
<td>CTL</td>
<td>Cell count/mL</td>
<td>The population of cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>INF-γ</td>
<td>mg/mL</td>
<td>The concentration of interferon-γ</td>
</tr>
<tr>
<td>Ks</td>
<td>mg/kg</td>
<td>The half-saturation constant</td>
</tr>
<tr>
<td>MHC class II</td>
<td>mg/mL</td>
<td>Major histocompatibility complex class II</td>
</tr>
<tr>
<td>N</td>
<td>Cell count/mL</td>
<td>The population of the natural killer cells</td>
</tr>
<tr>
<td>N*</td>
<td>Cell count/mL</td>
<td>Induced population of the natural killer cells</td>
</tr>
<tr>
<td>NK</td>
<td>Cell count/mL</td>
<td>The population of the natural killer cells</td>
</tr>
<tr>
<td>Tc</td>
<td>Cell count/mL</td>
<td>The population of cytotoxic T cells</td>
</tr>
<tr>
<td>Tc*</td>
<td>Cell count/mL</td>
<td>The population of the activated cytotoxic T cells</td>
</tr>
<tr>
<td>Th*</td>
<td>Cell count/mL</td>
<td>The population of the activated helper T cells</td>
</tr>
<tr>
<td>Th</td>
<td>Cell count/mL</td>
<td>The population of the helper T cells</td>
</tr>
<tr>
<td>VIC</td>
<td>Cell count/mL</td>
<td>The population of the virus infected cells</td>
</tr>
<tr>
<td>Vmax</td>
<td>%/D</td>
<td>Percent maximum induction rate of NK cells per day</td>
</tr>
<tr>
<td>K</td>
<td>----</td>
<td>The population constant of NK cells</td>
</tr>
<tr>
<td>k'</td>
<td>%kg/mg-D</td>
<td>The αth root of K</td>
</tr>
<tr>
<td>υ</td>
<td>%/D</td>
<td>Percent induction rate of the NK cells per day</td>
</tr>
<tr>
<td>d[INF-γ]/dt</td>
<td>mg/kg-D</td>
<td>The production rate of interferon-γ per kg per day</td>
</tr>
<tr>
<td>d[N*]/dt</td>
<td>%/D</td>
<td>Percent induction rate of NK cells</td>
</tr>
<tr>
<td>k1</td>
<td>kg/mg-D</td>
<td>The induction rate constant of NK cells by MGN-3</td>
</tr>
<tr>
<td>k2</td>
<td>kg/cell-D</td>
<td>The induction rate constant of NK cells by INF-γ</td>
</tr>
</tbody>
</table>

#### A2. The Mathematical Model Derivation

**A2.1. The Cytokine-Mediated Model (CM 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 Th cells to form activated Th cells (Effector cell, Th*) is mediated by the innate major histocompatibility complex (MHC) class II (Eq. 1), which in turn induces B cells, Te cells, and NK cells to produce induced B* cells (Eq. 2), induced CTL (Te* ) (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).

![Diagram of the Cytokine-Mediated Model](image)
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 Te* (Eq. 8):

VIC or ASC + N* –> Destroyed VIC or ASC ------7
VIC or ASC + Te* –> 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 (Cb) (Eq. 9), and the auto-catalytic activation of NK cells by INF-γ (Eq. 10) to give

N + Cb –> N* + INF-γ ------9
N + INF-γ –> N* ------10

A2.2. The Mathematical Mode

The induction of NK cells (N*) by MGN-3/Biobran and the subsequent auto-catalytic production of INF-γ is directly proportional to the squared root of INF-γ.

\[
\frac{d[N^*]}{dt} = k' [Cb] \sqrt{[INF-\gamma]} 
\]

And

\[
\frac{d[N^*]}{dt} = k_2 [N] [INF-\gamma] 
\]

Rearrangement of Eq. 16 yields

\[
\frac{d[N^*]}{dt}^2 = k [N] [Cb] [INF-\gamma] 
\]

Furthermore, 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

\[
\frac{d[N^*]}{dt}^2 = K [Cb] [INF-\gamma] 
\]

Here K = k [N]^2

Square root of Eq. 19 gives

\[
\frac{d[N^*]}{dt} = k' [Cb] [INF-\gamma] \frac{1}{2} 
\]

Or in general form

\[
\frac{d[N^*]}{dt} = k' [Cb] [INF-\gamma] \alpha 
\]

Where, k' = √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 (Cb) 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

\[
\mu = \mu_{max} \cdot \frac{Cb}{K_s + Cb} 
\]

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

\[
u = \mu N, \text{ and } V_{max} = \mu_{max} N 
\]

Thus

\[
u = V_{max} \cdot \frac{Cb}{K_s + Cb} 
\]

Where, \(\mu\) is the production rate of induced NK cells, \(\frac{dN^*}{dt}\); \(V_{max}\) 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

The authors wish to thank the financial grants respectively offered by the National Science Council (NSC 99-2320-B-038-011-MY3).

7. Conflict of Interest and Disclosure Statement

The authors have disclosed any conflict of interest.

8. References

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