The effects of cell-surface composition on natural killer cell activation: a modeling study

Thesis

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Abstract

Natural killer (NK) cells make up part of the innate immune system and are a good target for tumor immunotherapy as they do not require previous exposure to a pathogen in order to activate and attack it. The NK cell signaling pathway begins with the binding of surface ligands on an incoming cell to the surface receptors on the NK cell. The receptors can be divided into two categories: excitatory and inhibitory. The excitatory receptors promote activation when they bind to their ligands, while the inhibitory receptors act as a fail-safe to prevent activation when they bind to their ligands. In a model by Das (2010), the populations of ligands on incoming cells and receptors on natural killer cells are simplified to either excitatory or inhibitory.

Recent research indicates the presence of a third type of surface ligand that binds to both excitatory and inhibitory receptors on NK cells. Here we present a model that incorporates nonspecific ligands into the population of possible ligands on the surface of an incoming cell to study the dynamics of NK cell activation. Tumor immunotherapy is a form of cancer therapy that uses the patient’s body to combat cancer, a treatment that may be safer for the patient and have fewer side effects. Theoretical work in the natural killer cell signaling pathway will provide framework for new research in the area of immunotherapy.
Dedication

Dedicated to my supportive family.
Acknowledgements

I appreciate the support I received both from my thesis committee of Avner Friedman and Jayajit Das, and from the professors in the math department who gave me the knowledge to continue studying what I love.
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Publications


Fields of Study

Major Field: Mathematics
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Introduction: Natural Killer Cell Signaling

Natural killer (NK) cells have long been known to be active players in the body’s innate immune response to pathogenic invasion (Lanier, 2005). Their ability to attack incoming cells without previous exposure makes them good candidates for cancer tumor immunotherapy. NK cells use surface receptors encoded without somatic recombination to differentiate between healthy cells and abnormal ones, thus they are part of the innate immune system and are closely-related to T cells (Lanier, 2005). Similar to CD8+ T cells, NK cells attack incoming cells by secreting perforin and granzymes that break down the cell membrane of the opposing cell.

The intracellular biochemical pathway that causes natural killer cell activation begins with a cell in the extracellular matrix coming into contact with an NK cell and attaching to its surface by way of surface ligands binding to NK surface receptors. This interaction initiates a cascade of intracellular reactions within the NK cell that ultimately leads either to a peaceful disassociation or the activation of the NK cell and a subsequent secretion of biomolecules that attack the attached cell. Natural killer cells exhibit two mains types of surface receptors: excitatory and inhibitory. Excitatory receptors regulate NK activation while the inhibitory receptors act as a fail-safe to prevent the immune system from attacking itself (Lanier, 2005).
Although inhibitory receptors can have a range of extracellular design, they have a common signaling process intracellularly (Lanier, 2008). Once the ligand and receptor bind extracellularly, on the cytoplasmic side of the membrane the protein lymphocyte-specific protein tyrosine kinase (lck) binds to the activated receptor and aids in the either single or double phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM). Lipid anti-src homology phosphatase-1 (SHP-1) responds to this phosphorylation by dephosphorylating the receptor to suppress NK cell activation (Lanier 2008). Phosphorylated SHP-1 then deactivates a phosphorylated Vav protein (pVav) which continues a string of reactions that ultimately arrests NK cell activation (Stebbins et al, 2003). Studies show that, in the killer cell immunoglobulin-like receptor (KIR) class of ITIM’s, SHP-1 is both necessary and sufficient to mediate NK cell inhibition (Ono et al., 1997). Interestingly, SHP-1 can also be active in the activation of natural killer cells.

The activating receptors excite the cell beyond the threshold of activation so that it responds to incoming cells (Bryceson, et al., 2005). Activation receptors constantly express immunoreceptor tyrosine-based activation motifs (ITAM’s), which bind to lck and are subsequently once or twice phosphorylated in the cytosol when a ligand binds to the receptor. Once phosphorylated, the excitatory receptor binds to tyrosine kinases Syk and Zap70 (Lanier, 2008). Interestingly, the bound excitatory receptor binds to Syk/Zap70 when once phosphorylated, but this interaction has a 1000-fold weaker affinity than the interaction between Syk/Zap70 and a twice-phosphorylated bound receptor (Shoelson, 1997). The phosphorylated Syk/Zap70 molecules phosphorylate Vav, a guanosine nucleotide exchange factor protein, which plays an integral role in the
activation of NK cells. Increasing Vav activation significantly increases the cytotoxicity of natural killer cells (Billadeau et al, 1998) by phosphorylating extracellular signal-regulated kinase (ERK), which mediates the exocytosis of the granules into the interface between the NK cell and its target (Jiang et al., 2000). The activation of excitatory receptors on the surface of natural killer cells, therefore, initiates reactions that can initiate an immune response.

While the natural killer cell is clearly activated by the excitatory pathway and inactivated by the inhibitory pathway, there exist additional possible reactions that affect the dynamics of activation. While Syk/Zap70 can bind to lck-bound receptors, SHP-1 can also bind to once-phosphorylated activating receptors (Barrow and Trowsdale, 2006) indicating that the activation of excitatory receptors has the possibility of contributing to the inactivation of NK cells. The phosphorylation of the excitatory receptors upon binding to the corresponding ligand attracts both Syk/Zap70 and SHP-1 with a similar affinity, and, as a result, can either phosphorylate Vav, or dephosphorylate pVav (Shoelson SE, 1997). Additionally, research done by Hilary S. Warren et al. suggests that there exists a third population of ligands that can illicit both excitatory and inhibitory responses in NK cells (2001). Such ligands bind to excitatory and inhibitory receptors and cause differing responses based on their relative concentration in the ligand population. Warren et al. found that in the absence of excitatory and inhibitory ligands, for large concentrations of the nonspecific ligand CD158b mAb, NK cell activation was completely inhibited, while lower concentrations of the same ligand stimulated activation. The possibility of a new type of ligand that mediates natural killer cell
activation based on the number of ligands present on the cell surface should be integrated into a model of natural killer cell activation.

In his model of natural killer cell activation, Jayajit Das (2010) worked with populations of excitatory and inhibitory ligands on incoming cells to see how such populations affect the activation of NK cells. Das’s model identifies the intricate ways in which the excitatory and inhibitory pathways interact to either prevent activation or cause it. His model may be an over-simplification of the system, however, in two ways. Firstly, the model claims that both inhibitory and excitatory receptors bind to SHP-1 only when they are once phosphorylated. According to Shoelson, SHP-1 can bind to twice-phosphorylated receptors, a reaction that contributes to the absence of NK cell activation through a later dephosphorylation of pVav. We adjust for this possibility in our new model. Also, Das works with excitatory and inhibitory ligands exclusively, while here we investigate the possibility of non-specific surface ligands reacting with NK cells by incorporating a new population of ligands that bind to both the excitatory and inhibitory NK receptors.

Natural killer cells are an integral part of the body’s innate immune system, and thus investigating the methods by which NK cells attack target cells will give us a better idea of how to use the immune system to fight pathogens. The activation of NK cells has been studied with the simplification of only two populations of ligands present: excitatory and inhibitory. In an effort to establish a theoretical framework for future research of the activation of natural killer cells, we build a model of NK cell activation that incorporates excitatory, inhibitory, and nonspecific ligands into the surface composition of incoming
cells that will interact with natural killer cells and affect the dynamics of the subsequent intracellular signaling that leads to the response of the immune system.
A Model of Natural Killer Cell Activation

The model was built to run in BioNetGen, software designed by Michael L. Blinov to model biochemical systems. In Figures 1 and 2, reaction diagrams of natural killer cell inhibitory and excitatory pathways respectively indicate the complexes and reactions present in the model of natural killer cell activation where (*) indicates the presence of a phosphate ion, $R_i$ is a ligand, and I and T are inhibitory and excitatory receptors, respectively.
Figure 1: The pathway initiated by ligands binding to NK cell inhibitory surface receptors, where $i = \{\text{inhibitory, non-specific}\}$. 
Figure 2: The pathway initiated by ligands binding to NK cell excitatory surface receptors, where \( j \in \{ \text{excitatory, nonspecific} \} \).
The parameters used for the rate constants are taken from Das’s model of natural killer cell activation and are listed in Table 1. The binding rate constant for nonspecific ligands binding to their corresponding receptor was measured by Adams et al., who found that the ligand m157 bound to Ly49I receptors with an affinity of \( k_d = 0.2 \, \mu\text{M} \) (2007), which represents \( k_{\text{off}}/k_{\text{ion}} \). Thus the rate constant \( k_{\text{non}} \), the rate at which the nonspecific ligand binds to the inhibitory receptor was set to 0.04166\,\text{s}^{-1} \) to be consistent with the rate constant \( k_{\text{off}} \) from Das’s model, which was set to 0.025\,\text{s}^{-1}. The work by Shoelson (1997) indicates a factor of 1000 should separate the dissociation constant for once-phosphorylated and twice-phosphorylated ITIM’s and ITAM’s. To account for this behavior, \( k_{\text{offp1}} \) is three orders larger than \( k_{\text{offp2}} \).
<table>
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Table 1: Rate Constants for signaling pathways
In order to measure the activation of the model natural killer cell, a parameter $R$ is constructed out of the steady state presence of complexes that contribute either to excitation or inhibition of natural killer cells. The complexes in bold boxes are those that dephosphorylate pVav and thus discourage NK cell activation, while the dotted box complex phosphorylates Vav to activate Erk and contribute to NK cell activation. Thus the activity of the model natural killer cell is measured by relative changes in $R$, the ratio of Syk/Zap70-bound receptors to SHP-1-bound receptors defined in the following way:

$$R = \frac{T \ast \ast R_N Z \ast}{(T \ast R_N S + T \ast \ast R_N S + I \ast R_N S + I \ast \ast R_N S)}$$

The model natural killer cell signaling pathway integrates the work of several groups to provide a qualitative view of the role of ligand composition in the body’s immune response to incoming cells.
The Effects of Cell Surface Composition on NK Cell Activation

Using BioNetGen, we extend a ordinary differential equation model of NK activation that Das created to include fully-phosphorylated ITIM and ITAM complexes bound to SHP-1. Additionally, we introduce a new population of ligands that bind with equal affinity to both excitatory and inhibitory receptors on the natural killer cell surface. It has been suggested that such nonspecific ligands act as a safe-guard for the organism by preventing natural killer cell activation in high concentrations. However, studies show that middle concentrations of the same nonspecific ligand actually encourage NK cell activation. We incorporate these ligands into our model and vary the initial number of excitatory, inhibitory, and nonspecific ligands to test this observation from a theoretical standpoint.

Variations in Ligand and Receptor Presence on NK Cells and Incoming Cells

In Figure 3, we measure the effects of NK cell surface composition on the activation of an NK cell when an incoming cell is composed solely of a combination of nonspecific ligands and either excitatory or inhibitory ligands. As could be expected, incorporating nonspecific ligands onto a cell that is composed of all excitatory ligands decreases NK cell activation, while the addition of nonspecific ligands onto an incoming
cell that has only inhibitory ligands on its surface increases NK cell activation. This first trial shows that the cell responds to simple protocols in a reasonable way.

Figure 3: NK cell response to nonspecific ligands on a purely excitatory or inhibitory background

As natural killer cells have varying numbers of excitatory and inhibitory receptors on their cell membranes, we study the composition of the NK cell and the composition of the incoming cell through varying \( I, T, R_i, R_T, \) and \( R_N \). Figure 4 describes the results of these runs. When the incoming cell has an equal number of excitatory and inhibitory ligands (top), NK cells with a dominant number of excitatory receptors become increasingly active the more nonspecific ligands there are present on the incoming cell
surface. NK cells become more active as the number of nonspecific ligands on the incoming cell surface increases regardless of the NK receptor composition in the presence of an incoming cell with a dominant number of excitatory ligands on its surface (middle). Finally, in order to see any increase of NK cell activation with an increase in nonspecific ligands on the surface of an incoming cell with a dominant number of inhibitory ligands, the NK cell must have more than half of its receptors be excitatory. The behavior that arises when incoming cells have a combination of the three possible surface ligands varies depending on the composition of the receptors on the natural killer cell. In general, however, we see a monotonic increase or decrease in NK cell activation depending on the dominance of one type of receptor or ligand.
Figure 4: Behavior of NK cell with varying numbers of excitatory and inhibitory receptors in the presence of cells with varying types of surface ligands
Variations in NK Cell Activation in the Presence of Only Nonspecific Ligands

In their work with nonspecific ligands and the effects on NK cell activation, Warren et al. study the effects of incoming cells with only nonspecific ligands on the NK activation signaling pathway. According to their data, NK cells show increased activation as the number of nonspecific surface ligands on incoming cells increases. A maximum value of activation occurs, however, after which point activation declines when additional nonspecific ligands are introduced. Running simulations of the model NK cell with no excitatory or inhibitory ligands present on the incoming cell returns the results shown in Figure 5. Regardless of the receptor composition of the NK cell, we see an increase in activation of the immune system with an increase in nonspecific ligands on the surface of the incoming cell. Such behavior is an indication that perhaps additional behavior is happening in the system that requires a more intricate model to be observed.
Variations in the Binding Rate Constant $k_{\text{non}}$ Lead to Observed Behavior

Upon further study of the model natural killer cell, the activation dynamics described by Warren et al. can be replicated if the nonspecific ligands bind with a higher affinity to excitatory receptors than to inhibitory receptors. Figure 6 illustrates this result. As the affinity for excitatory receptors ($k_{\text{non}}$) is increased and the affinity for inhibitory receptors ($k_{\text{non}}$) is decreased, the excitatory receptors are filled before the inhibitory
receptors. Thus as the number of nonspecific ligands on the incoming cell surface increases, we see an increase in activation.

Figure 6: The NK cell activation effects of the nonspecific binding rate constant

When the number of nonspecific ligands approaches 500, however, all the excitatory receptors become filled leaving only the inhibitory receptors available for binding. Thus as the number of nonspecific ligands increases beyond the maximum number that may bind to the excitatory receptors, the NK cell activation at steady state drops. The opposite occurs if the affinity for the inhibitory receptors is greater than that for the excitatory receptors.
Future Directions for Natural Killer Cell Activation Pathway Research

In order to gain a better understanding of the mechanism behind natural killer cell activation, we construct a model that not only includes excitatory and inhibitory ligands shown to be present in the NK cell activation pathway (Lanier, 2005) but also implements a population of nonspecific ligands that bind to the excitatory and inhibitory receptors with equal affinity. We varied the initial values of the three populations of receptors and observed what these changes did to the parameter $R$, a measure of NK cell activation.

Our results indicate that the dynamics of NK cell activation depend on both the composition of the incoming cell’s ligands and the composition of the NK cell receptors. While the surface composition of natural killer cells is predetermined, it could be possible to block the activity of some or all receptors of NK cells, so understanding the changes in activation that arise from varying the populations of excitatory and inhibitory ligands and receptors provides a novel theoretical framework for further studies on natural killer cell activation.

Research by Warren et al. indicates that, when nonspecific ligands bind with the same affinity to both excitatory and inhibitory receptors, incoming cells with only nonspecific surface ligands cause a low activation of NK cells for small and large
populations of ligands, while middle populations of ligands produce a higher immune response. We tried to replicate this behavior in our model, but we were only successful in seeing this behavior when the nonspecific ligands had a preferential affinity for the excitatory receptors. Experiments in a laboratory would help to confirm or refute our results, but it is possible that we have oversimplified some of the intricate dynamics of NK cell activation that are required to obtain the same behavior that Warren documents. Future directions for the model should incorporate more reactions in the signaling pathway that will provide a clearer image of the NK cell signaling pathway.

Natural killer cells are a strong part of the body’s defense against pathogenic invasion. Research done in immunotherapy suggests NK cells would be strong candidates for new cancer treatments, and understanding the signaling pathway that leads to their activation is a strong first step towards treating cancer using the patient’s own immune system. This model provides a framework for immunotherapy research that may be extended and fine-tuned to further study the intricacies of natural killer cell activation.
References


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