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A MATHEMATICAL MODEL OF RECEPTOR-MEDIATED APOPTOSIS: DYING TO KNOW WHY *FASL* IS A TRIMER

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Abstract. The scientific importance of understanding programmed cell death is undeniable; however, the complexity of death signal propagation and the formerly incomplete knowledge of apoptotic pathways has left this topic virtually untouched by mathematical modeling. In this paper, we use a mechanistic approach to frame the current understanding of receptor-mediated apoptosis with an immediate goal of isolating the role receptor trimerization plays in this process. Analysis and simulation suggest that if the death signal is to be successful at low-receptor, high-ligand concentration, Fas trimerization is unlikely to be the driving force in the signal propagation. However at high-receptor and low-ligand concentrations, the mathematical model illustrates how the ability of FasL to cluster three Fas receptors can be crucially important for downstream events that propagate the apoptotic signal.

1. **Introduction.** Apoptosis or programmed cell death (PCD) is a critical process in normal tissue development [1]. It is the primary mechanism through which cells are removed when malfunctions arise from cell stress, cell damage, or conflicting cell division signals [2]. Maintaining the balance between programmed cell death and cell survival is fundamentally important since disturbing this equilibrium can lead to a number of pathological disorders. The core component of the cell suicide machinery is a family of proteases called caspases. The events culminating in caspase activation and the subsequent disassembly of the cell are the subject of intense study because of their role in many neurodegenerative disorders including Parkinson's and Alzheimers's diseases, autoimmune disorders, and tumorigenesis [3, 4]. In fact, many cancers are hypothesized to arise from and are difficult to eradicate because of the failure to respond to apoptotic signals [5, 6]. Understanding caspase regulation is intimately linked to the ability to rationally manipulate apoptosis for therapeutic gain [7].

Before a cell is committed to apoptotic death, a highly regulated cascade of events must occur culminating in the activation of cysteine-containing aspartate-specific proteases or caspases [7]. Caspases are constitutively expressed in normal cells in an inactive form termed procaspases and may be activated by either death receptor engagement or mitochondrial release of cytochrome c [2, 7, 8]. Until recently, the mechanisms of programmed cell death were ellusive; however, a more complete

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picture of the pathways to apoptosis has emerged and is outlined in [2, 3, 4, 7, 9, 10]. The major objective of this study is to use mathematical modeling to describe the current knowledge of receptor-mediated apoptosis and to enhance the current understanding of the role receptor aggregation plays in this process.

1.1. **Death-receptor engagement.** One of the most intensively studied pathways to cell death results from ligation of transmembrane death receptors belonging to the tumor necrosis factor-R1 (TNF-R1) family. Death receptors are engaged by specific ligands and transmit apoptotic signals via a homologous cytoplasmic sequence termed the death domain (DD). Caspase activation ensues within seconds of ligand binding to death receptors and cell death occurs within hours [2, 7].

The best characterized death receptor is Fas (also called CD95 or Apo1), which has FasL as its natural ligand. FasL is a type II membrane receptor that shares homology with a large number of tumor necrosis factor (TNF) family members. It is normally expressed by a small number of cell types, including activated lymphocytes and cells of the immune-privileged tissues (e.g., the eye, testis, brain, and spinal cord). Fas is a type I membrane receptor that, unlike FasL, is expressed constitutively in most tissues and is dramatically up-regulated at sites of inflammation. FasL is a homotrimeric molecule capable of binding up to three Fas receptors. Because death domains have a propensity to associate with one another, Fas ligation leads to clustering of the receptors' death domains (see Fig. 1) [2, 11]. An adaptor protein called FADD (Fas-associated death domain), which contains its own death domain as well as a death effector domain, is then recruited and binds to the clustered death domains of the Fas receptors [12, 13, 14]. Experimental evidence suggests that FADD binding is associated only with aggregated (the signaling form of Fas), not with monomeric Fas [15]. Once FADD is in place, the close proximity model suggests that FADD brings two or more procaspase-8 monomers near enough to activate each other by proteolytic cleavage [16, 17]. Caspase-8 is an initiator caspase; its activation stimulates the downstream activation of executioner-level caspases (such as caspases-3, -6 and -7) which results in the degradation of cellular structures, and the apoptotic death of the cell soon follows [2]. Although the apoptotic cascade can be similarly induced by the engagement of several other death receptors, including DR4 and DR5 by TRAIL (tumor necrosis factor (TFN)-related apoptosis-inducing ligand) [18, 19], this work will concentrate on the well known Fas/FasL system. Figure 1 describes the signaling of apoptosis by Fas/FasL engagement.

The death receptor engagement pathway of caspase activation is but one mechanism by which individual cells can be instructed to undergo apoptosis. Another pathway that leads to the same result, in the absence of an external signal, is the mitochondrial release of cytochrome c when cells are stressed or experience DNA damage [8]. Modeling this pathway will be the subject of future work.

In the following sections, a mathematical model of the cell surface binding events as well as the intracellular signaling processes that lead to caspase-mediated apoptosis is developed and analyzed. An immediate goal of this model is to investigate and assist in understanding the role Fas trimerization has on downstream events leading to cell death. Future work will focus on specific questions concerning the role of apoptotic failure in the genesis and drug resistance of the common childhood cancer, neuroblastoma.

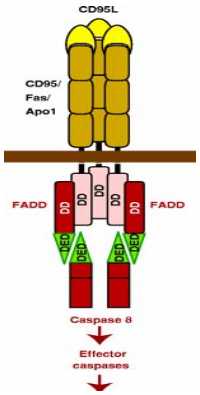

FIGURE 1. Fas/FasL (also known as CD95/CD95L) engagement pathway leading to caspase activation and apoptosis. FasL binds up to three Fas receptors, which results in death domain clustering. FADD then binds to the clustered Fas death domains and recruits procaspase-8 monomers that activate each other. Activated caspase-8 then stimulates the downstream activation of execution level caspases and cell death results. Diagram reproduced from [2].

2. **Mathematical model development.** The model for the Fas/FasL engagement pathway developed here is based on experiments that show FasL (L) to be a homotrimeric molecule capable of binding up to three Fas (R) receptors [2, 11]. Because Fas ligation leads to receptor aggregation, we propose the following multivalent-binding and receptor cross-linking model [20, 21, 22, 23]. To our knowledge, this the first time such a cross-linking model has been applied to the Fas/FasL system. A reaction diagram for describing the binding events associated with Fas/FasL can be drawn as follows:

$$L + R \underset{k_r}{\overset{3k_f}{\rightleftarrows}} C_1 + R \underset{2k_{-x}}{\overset{2k_x}{\leftrightarrows}} C_2 + R \underset{3k_{-x}}{\overset{k_x}{\leftrightarrows}} C_3 \tag{1}$$

where L represents free FasL; R is the number of free receptors; and C_1, C_2, C_3 are the number of singly, doubly, and triply bound Fas/FasL complexes, respectively. The parameters k_f and k_r are the forward and reverse reaction rates, and k_x and k_{-x} are the forward and reverse cross-linking rates. The integer factors multiplying the reaction rates account for statistical factors: there are three ways (either site) a trivalent ligand can become singly bound to a free receptor, two ways a singly bound receptor can become doubly bound, and so on [20]. Invoking the law of mass action yields, the following differential equations for the concentration of free FasL (l) and the concentrations of singly (c_1), doubly (c_2), and triply (c_3) bound receptor-ligand complexes. Assuming receptor synthesis and trafficking are negligible on the time scale of interest results in an algebraic equation for the free receptor concentration, r, in terms of the total receptor concentration r_T and the total concentration of bound receptor-ligand complexes.

$$\begin{aligned}
\frac{dl}{dt} &= -3k_f lr + k_r c_1 \qquad\qquad l(0) = L_0,\\
\frac{dc_1}{dt} &= 3_f lr - k_r c_1 - 2k_x c_1 r + 2k_{-x} c_2 \qquad\qquad c_1(0) = 0,\\
\frac{dc_2}{dt} &= 2k_x c_1 r - 2k_{-x} c_2 - k_x c_2 r + 3k_{-x} c_3 \qquad\qquad c_2(0) = 0,\\
\frac{dc_3}{dt} &= k_x r c_2 - 3k_{-x} c_3 \qquad\qquad c_3(0) = 0,\\
r_T &= r + c_1 + 2c_2 + 3c_3.
\end{aligned} \tag{2}$$

It is assumed that these binding events occur rapidly, yielding steady-state concentrations of the singly, doubly, and triply bound Fas receptors. We will consider this steady-state profile to be the death signal required to initiate the downstream events leading to caspase activation.

Once clustered, the death domains of the Fas receptor associate with each other [2] leaving two unassociated sites that can associate with the death domains of FADD. In other words, the cytoplasmic tail of the Fas receptor itself becomes a bivalent receptor for the intracellular adaptor protein, FADD (see Fig. 1).
The following system of differential equations describes FADD recruitment:

$$\begin{aligned}
\frac{d\tilde{c}_2}{dt} &= -2\kappa_f f\tilde{c}_2 + \kappa_r d_{12} \qquad \tilde{c}_2(0) = \tilde{c}_2^0,\\
\frac{d\tilde{c}_3}{dt} &= -2\kappa_f f\tilde{c}_3 + \kappa_r d_{13} \qquad \tilde{c}_3(0) = \tilde{c}_3^0,\\
\frac{dd_{12}}{dt} &= 2\kappa_f f\tilde{c}_2 - \kappa_r d_{12} - \kappa_x f d_{12} + 2\kappa_{-x} d_{22} \qquad d_{12}(0) = 0,\\
\frac{dd_{22}}{dt} &= \kappa_x f d_{12} - 2\kappa_{-x} d_{22} \qquad d_{122}(0) = 0,\\
\frac{dd_{13}}{dt} &= 2\kappa_f f\tilde{c}_3 - \kappa_r d_{13} - \kappa_x f d_{13} + 2\kappa_{-x} d_{23} \qquad d_{13}(0) = 0,\\
\frac{dd_{23}}{dt} &= \kappa_x f d_{13} - 2\kappa_{-x} d_{23}
\end{aligned} \tag{3}$$

FADD and Fas conservation implies the following algebraic relations:

$$\begin{aligned}
f_T &= f + d_{12} + 2d_{22} + d_{13} + 2d_{23}\\
\tilde{c}_2^0 &= \tilde{c}_2 + d_{12} + d_{22}\\
\tilde{c}_3^0 &= \tilde{c}_3 + d_{13} + d_{23}.
\end{aligned} \tag{4}$$

In Equations (3) and (4), $\tilde{c}_2$ and $\tilde{c}_3$ represent the concentrations of doubly and triply clustered Fas death domains and d_{ij} are the concentrations of j clustered death domains with i FADD molecules attached. The parameters κ_f and κ_r are the forward and reverse reaction rates associated with a single FADD molecule binding to the death domain on Fas, and κ_x and κ_{-x} are the forward and reverse binding rates once, at least one FADD molecule has attached. The total intracellular FADD concentration is f_T, and the initial concentrations of the doubly and triply clustered Fas death domains are denoted by c_2^0 and c_3^0, respectively. Note that this model assumes that the doubly and triply bound Fas receptors can carry the signal for downstream events. We will use numerical simulations to investigate how relaxing this assumptions affects caspase activation.

3. Analysis and numerical simulations.

3.1. Fas engagement and clustering.

Many interesting and important cell functions involve multivalent binding phenomena similar to the one described above; for example, the binding of antigen-antibody complexes to monovalent Fc receptors on white blood cells and viral attachment to cell surfaces [20]. Given the need for quantitative understanding of these complicated process, much effort has gone into the development and analysis of mathematical models for multivalent receptor-ligand binding. In the next sections we build on these traditional analyses to gain insight into the Fas-FADD system.

3.1.1. *Negligible FADD Depletion.* The model given in (2) describes the first steps in the receptor engagement pathway of programmed cell-death initiation. Studying this system in isolation can provide important information regarding the dynamics of Fas-FasL binding and the sensitivity of the predicted behavior to the model parameters. For example, it has been shown [22] that if ligand depletion is negligible (i.e., $l = L_0$), the equilibrium number of singly, doubly, and triply bound receptors is:

$$\begin{aligned} c_{1eq} &= 3\left(\frac{L_0}{K_D}\right) r_{eq} \\ c_{2eq} &= 3K_x\left(\frac{L_0}{K_D}\right) r_{eq}^2 \\ c_{3eq} &= K_x^2\left(\frac{L_0}{K_D}\right) r_{eq}^3. \end{aligned} \tag{5}$$

In Equation (5), $K_D = k_r/k_f$ is the equilibrium dissociation constant, and $K_x = k_x/k_{-x}$ is the equilibrium crosslinking constant. The equilibrium number of free receptors, r_{eq}, must be determined from the following cubic equation which has at most one positive root,

$$cr_{eq}^3 + br_{eq}^2 + ar_{eq} - r_T = 0, \qquad c = 3K_x^2\left(\frac{L_0}{K_D}\right), b = 6K_x\left(\frac{L_0}{K_D}\right), a = 1+3\left(\frac{L_0}{K_D}\right). \tag{6}$$

From this information, the total number of cross-links at equilibrium can be computed; $C_{XT} = c_{2eq} + 2c_{3eq}$ and a cross-linking curve that plots C_{XT} versus the ligand concentration can be constructed. This type of diagram can determine the range of ligand concentrations for which clustering is successful. From Figure 2 it is clear that successful clustering occurs for an intermediate range of scaled ligand concentrations, and as expected, increasing the nondimensional cross-linking constant $\kappa = K_x r_T$ leads to an enhanced receptor aggregation.

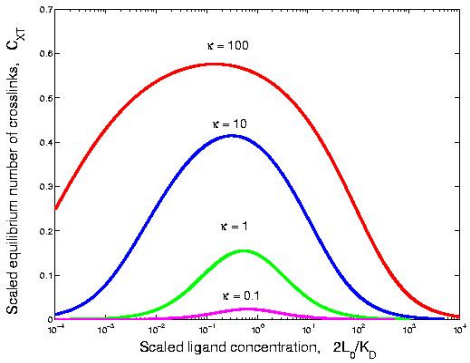

FIGURE 2. Equilibrium cross-linking curve plotting the scaled equilibrium number of cross-links (C_{XT}) as a function of the scaled ligand concentration ($2L_0/K_D$) for various choices of the cross-linking equilibrium constant κ.

Table 1 lists the nondimensional ligand concentration, $2L_0/K_D$, which results in the maximum percentage of cross-links for $\kappa = 0.1, 1.0, 10, 100$. As κ increases the ligand concentration required for maximal cross-linking decreases. Table 1 also shows that the percentage of the total cross-links at equilibrium that are triply bound increases as κ increases. Therefore, if the death signal is to be successful at low receptor concentration (i.e., $\kappa = K_x R_T$ small) and high ligand concentration, Fas trimerization is unlikely to be the driving force in the signal propagation, as only a very small percentage of the total number of crosslinked receptors are in the triply bound form. However at high receptor and low-ligand concentrations, the majority of the total number of cross-links is made up of triply bound complexes and Fas trimerization could be important for the downstream cascade of events.

TABLE 1. *Nondimensional ligand concentraion, maximum cross-linking, and percentage of triply bound complexes for various choices of the cross-linking constant* κ.

κ	Ligand Concentration	Cross-links	Triply Bound Complexes
0.1	0.63	2.4%	3%
1.0	0.50	16%	21%
10	0.29	41%	57%
100	0.14	58%	83%

3.1.2. *Role of Fas trimerization at the level of Fas/Fas cross-linking.* If Fas trimerization should fail completely– that is, if FasL behaves as a dimer and none of the

cross-links can triply bind– $\kappa = 1.0$ would yield, at most, 8.5% cross-linking compared to 16% when triply bound clusters form (most of the 16% are doubly bound complexes). Further, if κ increases to 100, failure to produce triples would yield, at most, 41% total cross-linking, compared to 58% when triply bound complexes are possible (in this case, most of the 58% are triply bound complexes). Therefore when κ is large, the fact that triply bound clusters can form results only in a moderate increase in the total number of cross-links at equilibrium; however, when κ is small, the ability of FasL to associate three Fas receptors can double the number of cross-links formed.

3.1.3. *Significance of FasL depletion.* The above steady-state solutions are based on the assumption that ligand depletion is negligible, that is, $l = L_0$. If this assumption is relaxed, the steady-state solutions become

$$\begin{aligned} c_{1eq} &= 3\left(\frac{L_0}{K_d}\right) r_{eq} l_{eq} \\ c_{2eq} &= 3K_x\left(\frac{L_0}{K_D}\right) r_{eq}^2 l_{eq}, \\ c_{3eq} &+ K_x^2\left(\frac{L_0}{K_D}\right) r_{eq}^3 l_{eq}, \end{aligned} \tag{7}$$

where the equilibrium number of free receptors, r_{eq}, must be determined from the following equation:

$$\kappa r^* r_{eq}^4 + c r_{eq}^3 + b r_{eq}^2 + a r_{eq} - R_T = 0, \tag{8}$$

where $c = \kappa r^*(3-\kappa) + 3K_x^2\left(\frac{L_0}{K_D}\right), b = 3r^*(1-\kappa) + 6K_x\left(\frac{L_0}{K_D}\right), a = 1 + 3\left(\frac{L_0}{K_D} - 3r^*\right)$, and $r^* = \frac{R_T}{K_D}$. Finally, the concentration of free ligand at equilibrium is given by

$$l = \frac{1}{1 + 3r^* r_{eq} + 3\kappa r^* r_{eq}^2 + \kappa^2 r^* r_{eq}^3}. \tag{9}$$

The effects of ligand depletion are simulated in figures 3 and 4. In this case, as $r^* = \frac{R_T}{K_D}$ increases, the range of ligand concentrations for which clustering is successful decreases and maximum cross-linking occurs for higher ligand concentrations.

From this point on, the equilibrium number of crosslinks as determined by the Fas-FasL model (2) described and analyzed above will be considered the signal for the initiation of the apoptotic cascade; that is, the death signal. Most importantly, these equilibrium values will be used as initial conditions for the intracellular events of FADD recruitment and caspase-8 activation.

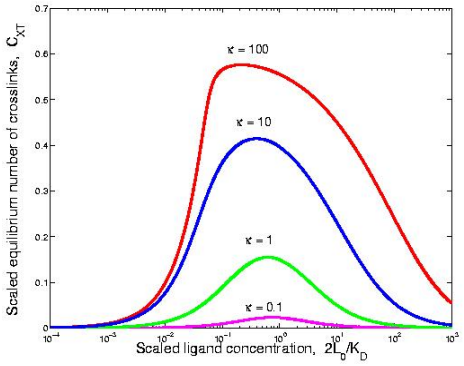

FIGURE 3. Equilibrium cross-linking curve plotting the scaled equilibrium number of cross-links (C_{XT}) as a function of the scaled ligand concentration ($2L_0/K_D$) for various choices of the cross-linking equilibrium constant0 κ, when ligand depletion is considered. In this figure $r^* = 0.1$.

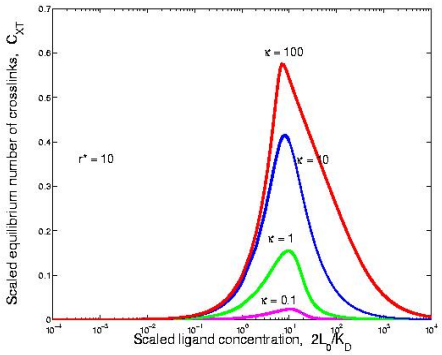

FIGURE 4. Equilibrium cross-linking curve plotting the scaled equilibrium number of cross-links (C_{XT}) as a function of the scaled ligand concentration ($2L_0/K_D$) for various choices of the cross-linking equilibrium constant κ, when ligand depletion is considered. In this figure $r^* = 10$.

3.2. **FADD recruitment.** The aggregation of Fas results in a conformational change in the receptors' death effector domains, which are part of their cytoplasmic tails. The intracellular adaptor protein, FADD (Fas-associated death domain), is then recruited and binds to the clustered death domains of the Fas receptors [12, 13, 14]. Therefore once the death signal is received; that is once Fas clustering is in equilibrium, FADD recruitment begins and the initial values of the clustered death domains are known. If FADD depletion is negligible, Equation (3) suggests the following steady states for FADD recruitment:

$$\begin{aligned}
\tilde{c}_{2eq} &= \frac{\tilde{c}_2^0}{1+2\alpha+2\alpha\beta} \\
\tilde{c}_{3eq} &= \frac{\tilde{c}_3^0}{1+2\alpha+2\alpha\beta} \\
d_{12} &= \frac{2\alpha\tilde{c}_2^0}{1+2\alpha+2\alpha\beta} \\
d_{22} &= \frac{2\alpha\beta\tilde{c}_2^0}{1+2\alpha+2\alpha\beta} \\
d_{13} &= \frac{2\alpha\tilde{c}_3^0}{1+2\alpha+2\alpha\beta} \\
d_{23} &= \frac{2\alpha\beta\tilde{c}_3^0}{1+2\alpha+2\alpha\beta}
\end{aligned} \tag{10}$$

where $\alpha = F_0/K_{\mathfrak{D}}$, $\beta = F_0/K_{\mathfrak{X}}$, $K_{\mathfrak{D}} = \kappa_r/\kappa_f$ and $K_{\mathfrak{X}} = \kappa_{-x}/\kappa_x$. Also, the initial conditions, $\tilde{c}_2^0$ and $\tilde{c}_3^0$ are determined from the steady-state analysis given in section 3.1.1. From the steady state solutions in Equation (10), it is clear that as $\beta \to \infty$, the total amount of bound FADD, d_T, approaches $2c_2^0 + 2c_3^0$. Further, as $\alpha \to \infty$, $d_T \to (c_2^0 + c_3^0)\frac{1+2\beta}{1+\beta}$.

Figure 5 illustrates the steady-state profiles of FADD recruitment when depletion is negligible. The initial conditions for clustered Fas death domains are taken to be the maximum levels (determined in section 3.1.1, see fig. 2) when $\kappa = 100$ for figure 5 and when $\kappa = 1.0$ for figure 5B. This provides insight into two different scenarios for FADD recruitment. From figure 5, it is clear that for a fixed constitutive level of FADD, F_0, not only is the dissociation constant important for successful recruitment (as determined by varying the nondimensional parameter, α), but the nondimensional cross-linking constant β is also a critical parameter. Therefore even if Fas aggregation is successful as is the case for initial conditions in figure 5, FADD recruitment can fail if its cross-linking constant is not sufficiently large. In figure 5B, the initial configuration of doubly and triply bound Fas is changed from $c_2^0 = .1021$, $c_3^0 = .2371$ to $c_2^0 = .1222$, $c_3^0 = .0164$ (this corresponds to varying κ from 100 to 1.0), and this also results in a marked reduction in FADD binding.

3.2.1. *Effect of dimeric Fas being unable to recruit FADD.* The effect of relaxing the assumption that both doubly and triply bound Fas can be associated with a FADD molecule is explored in figure 5. In this case, approximately 20% less FADD is bound at equilibruim. In section 3.3 we will show how this affects caspase-8 activation.

3.2.2. *Role of Fas trimerization at the level of FADD recruitment.* To determine the role of Fas trimerization at the level of FADD recruitment, figure 5 plots the steady-state profiles of bound FADD as a function of FADD concentration when trimerization fails completely. Here, $\kappa = 100$ or 1.0 corresponding to $c_2^0 = 0.41$ or 0.085 and $c_3^0 = 0$. Notice that when only dimers form and $\kappa = 100$, there is actually more bound FADD at equilibrium; however, when $\kappa = 1.0$ this situation is reversed.

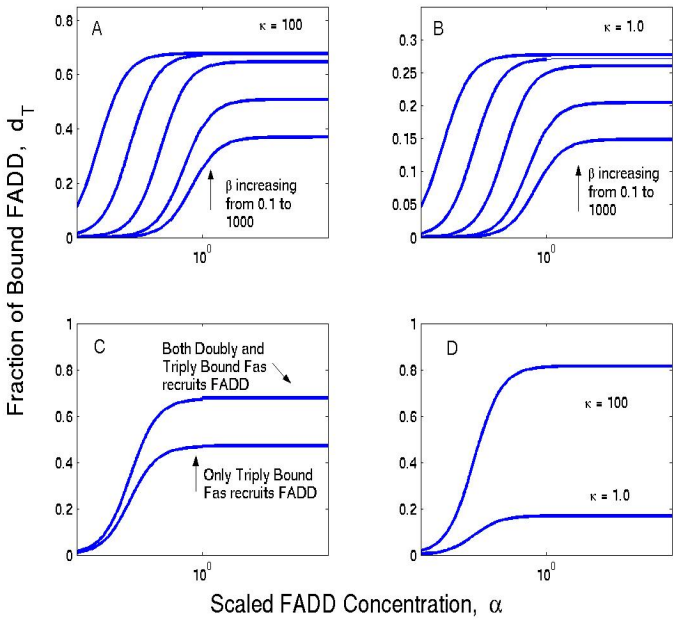

FIGURE 5. Equilibrium FADD binding curve plotting the scaled equilibrium concentration of bound FADD (d_T) as a function of the scaled FADD concentration ($\alpha = F_0/K_{\mathfrak{D}}$) for various choices of the cross-linking equilibrium constant $\beta = F_0/K_{\mathfrak{X}}$ when FADD depletion is ignored. (a) $\kappa = 100$ so that more than 50% of the Fas receptors are cross-linked; this gives $c_2^0 = .1021$, $c_3^0 = .2371$. In (b) $\kappa = 1.0$, and less than 20% of the Fas receptors are cross-linked; this gives $c_2^0 = .1222$, $c_3^0 = .0164$. (c) Comparing equilibrium FADD binding when only Fas trimers are capable of recruiting FADD: $\kappa = 100$ and $\beta = 100$. (d) Consequences of only Fas dimers forming: $\beta = 100$.

Similar analytical solutions can be obtained for the case when FADD depletion is considered. For a given β, the overall effect of FADD depletion is to decrease the maximum concentration of bound FADD and to shift to the right the FADD concentration corresponding to the change in concavity (results not shown).

3.3. **Caspase-8 activation.** Two mechanisms of procaspase-8 activation are considered: (i) autocatalysis and (ii) the close proximity model. The latter assumes that two or more procaspase-8 molecules bind to the death-effector domain of FADD (multivalent as a receptor) and once in place, activate each other [16, 17]. A reaction diagram for this model is given in (11); however, note that these reactions are additional to those described by the equations in (3).

$$
\begin{aligned}
P + D_{21} &\leftrightarrows^{k_{-p}}_{k_p} N_{11} \\
P + D_{22} &\leftrightarrows^{k_{-p}}_{k_p} M_{21} \\
M_{21} + P &\leftrightarrows^{k_{-c}}_{k_c} M_{22} \rightarrow^{k_a} D_{22} + 2E \\
P + D_{31} &\leftrightarrows^{k_{-p}}_{k_p} N_{11} \\
P + D_{32} &\leftrightarrows^{k_{-p}}_{k_p} M_{21} \\
M_{21} + P &\leftrightarrows^{k_{-c}}_{k_c} M_{22} \rightarrow^{k_a} D_{32} + 2E \\
P + P &\rightarrow^{\bar{k}_a} 2E
\end{aligned}
\tag{11}
$$

Diagram (11) describes free procaspase-8 molecules P binding to death complexes composed of doubly bound Fas receptors with one (D_{21}) or two (D_{22}) FADD proteins attached. The former results in a terminal complex N_{11} whereas the latter forms complex M_{21}, which can accept an additional procaspase-8 molecule to form M_{22}. The two procaspase-8 molecules that have been brought into proximity via binding now activate each other, releasing two molecules of activated caspase-8 (E) and an unoccupied D_{22} receptor. The reaction diagram for the binding procaspase-8 two death complexes that consists of triply bound Fas receptors with one (D_{31}) or two (D_{32}) FADD proteins follows in a similar manner. Finally two free molecules of procaspase-8 may activate each other, producing two caspase-8 molecules.

Figure 6 simulates receptor-mediated caspase-8 activation as described in (11) with parameters listed in table 2. The death signal (steady state FAS clustering) is received at time zero at which point the fraction of active caspase-8 is 0.5%. Once the death signal is received, about50% activation occurs within five hours when trimerization is successful. Changing κ from 100 to 1.0 results in over a four hour delay in activation. The effect of FADD binding success on caspase activation is illustrated in Figure 6B. When FADD binding is disrupted (β decreasing) caspase activation is significantly reduced, and the apoptotic signal is either delayed or terminated.

TABLE 2. *Parameters values used in the caspase-8 activation simulations.*

Parameter	Value
k_p	5×10^6
k_{-p}	0.4
k_c	1.5×10^8
k_{-c}	1.0
k_a	1×10^4
$\bar{k}_a$	1.0×10^{-4}
α	3×10^6
β	0.2

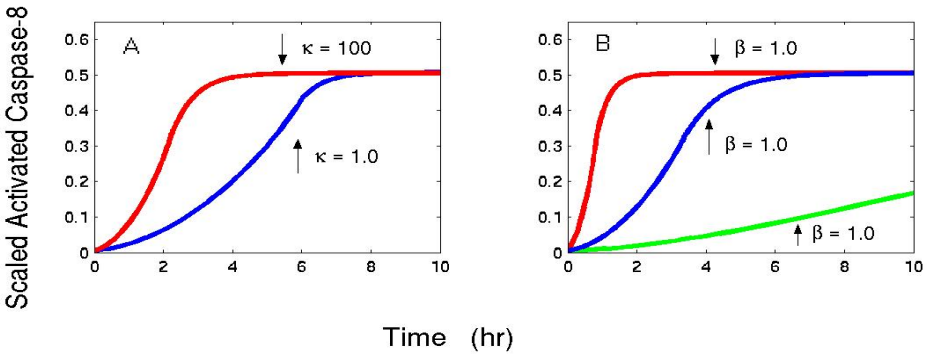

FIGURE 6. Fraction of active caspase-8 as a function of time. (a) The effect of FAS trimerization: In the upper curve, $\kappa = 100$ so that over 50% of the Fas receptors are cross-linked; this gives $c_2^0 = .1021$, $c_3^0 = .2371$ and in the lower curve $\kappa = 1.0$ and less than 20% of the Fas receptors are cross-linked; this gives $c_2^0 = .1222$, $c_3^0 = .0164$. (b) The effect of FADD binding disruption: As β decreases, caspase-8 activation is significantly decreased.

4. **Discussion.** In this paper we have developed a mathematical model of the receptor-mediated pathway to initiator caspase activation associated with the apoptotic cascade. Our objective has been to gather essential elements of the current understanding of caspase activation into a corresponding mathematical description that can be used to investigate the relative importance of receptor aggregation on the downstream events that lead to cell death. The mathematical model is based on receptor-ligand binding kinetics and mass balances. Although, the majority of the current knowledge is qualitative, this mathematical approach offers a conceptual structure that allows for further exploration and examination of the available information.

The death signal is initiated when the homotrimeric ligand FasL binds and clusters Fas receptors. Our analysis shows, that receptor aggregation will be most successful for a small range of ligand concentrations [22] and that as the nondimensional cross-linking constant increases, so does the percentage of total clustered triply bound receptors. This implies that if the death signal is to be successful at low-receptor, high-ligand concentration, Fas trimerization is probably not the critical factor in propagating the death signal, as only a very small percentage of the total number of the receptor-ligand complexes are in the triply bound form.

However at high receptor and low ligand concentrations, most of the complexes are triply bound and should the death signal fail downstream, Fas trimerization in not likely to be the reason.

Fas aggregation results in a conformational change in the receptors' death effector domains, which enables the adaptor protein FADD to bind. Therefore even if Fas aggregation is successful, FADD recruitment can still fail if its crosslinking constant is not sufficiently large. It is not clear whether the doubly bound FasL/Fas complex can associate with FADD. Our model predicts that approximately 20% less FADD is bound at equilibruim if FADD can only attach to triply bound complexes . Further, decreasing the initial concentration of fully aggregated receptors results in a delay in caspase activation, whereas disruption of FADD binding critically reduces levels of activated caspase-8 and can result in the termination of the death signal.

There are other factors that can induce caspase activation that are not related to receptor-ligand binding. These include cell stress, cell damage, and cytotoxic drugs [24, 25]. There are also several well-known, intracellular decoy proteins which compete for crucial binding sites at several junctures in the apoptotic cascade [26, 27]. A future investigation will explore the stress- and drug-induced pathways to apoptosis and consider the effect of inhibitory molecules, with the goal of investigating the role of apoptosis in tumorigenesis as well as the development of drug resistance that is associated with many cancers.

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