IMMUNE SET POINT: SET POINTS VS CHECK POINTS?

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We examine some concepts put forth regarding set point principles in immune response. We provide some additional insight options and expand the discussion to consider other options.

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1 INTRODUCTION

There has been a multiplicity of applications of such therapeutics as monoclonal antibodies which are used as "checkpoint inhibitors" to treat a variety of cancers. These therapeutics are proteins which can block the action of inhibitors on T cells which if activated and prevent the T cell from attacking the cancer cell. We have seen the proliferation of these therapeutic approaches in melanoma, lung cancer and even prostate cancer.

The main driver for this analysis is the recent work of Chen and Mellman. In a recent paper these two authors state:

*Immunotherapy is proving to be an effective therapeutic approach in a variety of cancers. But despite the clinical success of antibodies against the immune regulators CTLA4 and PD-L1/PD-1, only a subset of people exhibit durable responses, suggesting that a broader view of cancer immunity is required. Immunity is influenced by a complex set of tumour, host and environmental factors that govern the strength and timing of the anticancer response. Clinical studies are beginning to define these factors as immune profiles that can predict responses to immunotherapy. In the context of the cancer immunity cycle, such factors combine to represent the inherent immunological status — or '**cancer–immune set point'** — of an individual.*

The concept of a "set point" is in our opinion rather poorly used. The construct, if properly understood, means that there is some point at which T cell activators and inhibitors either permit activation and effective T cell immunotherapeutic action or inhibit that. Namely there is some set of activations less inhibitions which all T cells to perform and under that "set point" they no longer function.

If such a concept has physical meaning, then the authors state:

Although largely conceptual, the idea of a set point provides a framework to help organize the torrent of clinical and biomarker data that will emerge over the coming months and years. The number of targets that could prove effective for cancer immunotherapy is great; the number of potential combinations of therapeutic agents that are directed against these targets (or combinations of such agents with conventional standard-of-care agents) is even greater. The development of some cancer therapies may be largely empirical, but it can be guided by considering, even in general terms, the elements that comprise cancer immunity

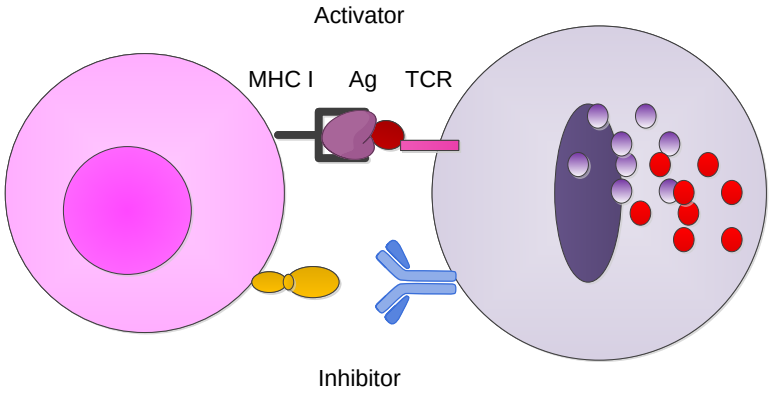
Thus, our objective in this paper is to examine this set point concept and explore its dimensions. Specifically, we examine how it may be used for therapeutic uses.

2 SOME PRINCIPLES

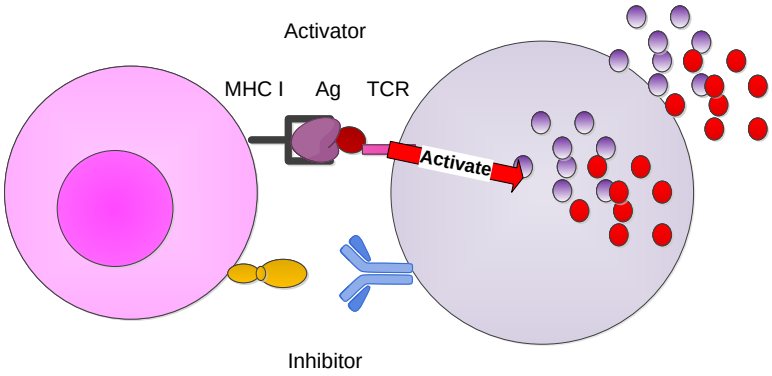
To best understand some of the principles we examine some simplistic model.

2.1 ACTIVATOR/INHIBITORS

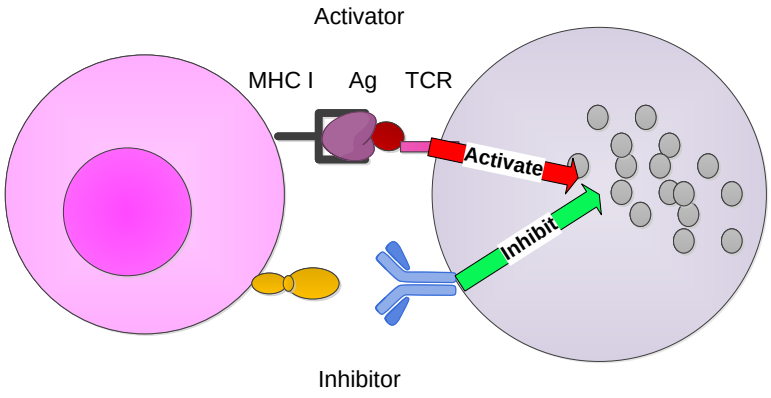
Let us begin with a simplistic but representative set of examples. We know that a CD8 T cell has a receptor, the TCR, T cell receptor, and it examines an antigen presenting cell, APV, which presents an antigen on its MHC I protein. This process is essentially an activator process. If that were all which was needed, then the T cell would be activated and sent out its cytokines and destroy the cell. However, there are also inhibitor ligands which activate inhibition in the T cell. We show these two below.



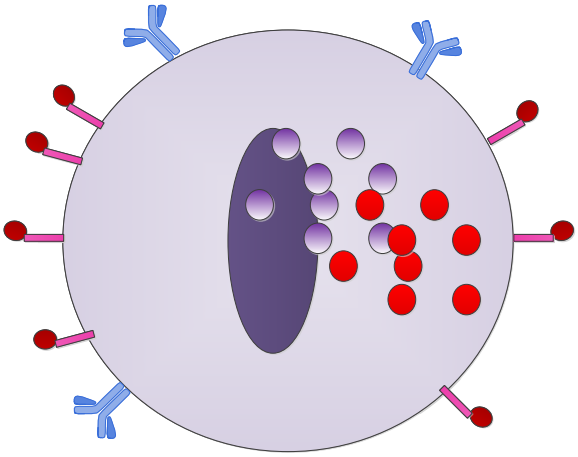
Now if the T cell is activated and the inhibition is not then we get the T cell sending out cytokines and killing the offending cell.



However if the T cell has an inhibitor also connected then the inhibitor sends out an internal T cell signal which stops the release. The presenting cell survives.



Now in reality the T cells do not have just one receptor. It may have a multiplicity over the surface. Thus there are a multiplicity of activators and inhibitors. It is a multiplicity amongst the same type as well a multiplicity of types. In fact the T cell may be just covered with receptors searching out antigens.



Thus the first layer of complexity of the immune response is not a simple activator/inhibitor complex but a mass of receptors and ligands interacting in a complex manner. The question then is; at what point does the T cell go from active to inactive and back again? In fact we may ask if there is some hysteresis effect. If so can we therapeutically take advantage of it.

2.2 LIGAND/RECEPTOR DYNAMICS

Ligands and receptors are basically two separate chemical elements. The binding of them is also essentially a chemical process whereby the ligand finds the correct location on the receptor to bind. It is in many ways like any chemical reaction. As such there is a reaction rate whereby the ligand and receptor combine, but equally there is the reverse reaction, the breaking apart of a bond.

In the work by Stone et al the authors note:

The interaction between the T-cell receptor (TCR) and its peptide–major histocompatibility complex (pepMHC) ligand plays a critical role in determining the activity and specificity of the T cell. The binding properties associated with these interactions have now been studied in many systems, providing a framework for a mechanistic understanding of the initial events that govern T-cell function. There have been various other reviews that have described the structural and biochemical features of TCR: pepMHC interactions.

Here we provide an overview of four areas that directly impact our understanding of T-cell function, as viewed from the perspective of the TCR: pepMHC interaction:

(1) relationships between T-cell activity and TCR: pepMHC binding parameters,

(2) TCR affinity, avidity and clustering,

(3) influence of coreceptors on pepMHC binding by TCRs and T-cell activity, and

(4) impact of TCR binding affinity on antigenic peptide specificity.

Namely there is a reaction such as:

$$TCR + pepMHC \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} TCR : pepMHC$$

Now they conclude:

The binding properties of TCRs for their pepMHC ligands are critically important in the function of T cells, leading to outcomes that can involve T-cell selection in the thymus or full peripheral T-cell responsiveness or homeostatic T-cell proliferation in the periphery. The processes are even more complicated because the same TCR could interact with multiple pepMHC ligands on the same antigen-presenting cell, each with heterogeneous binding properties. These reactions would result in a complex integration of signals that ultimately determine the nature of the T-cell response.

While there have been numerous studies to elucidate the precise binding parameters that correlate with different T-cell activities, various questions remain unanswered (in part because of the technical difficulties associated with performing binding experiments on low-affinity reactions). Further understanding of the TCR binding properties that generate defined signals is

important, not only from a basic science perspective but also toward developing optimal strategies that improve T-cell responses to foreign antigens and tumour antigens.

Thus, one must be careful in developing an immune set point theory to be cautious about the affinity issues as discussed above.

2.3 OVERALL PROCESS

We have examined the complex process fundamentally as a build. Specifically:

1. Activation: When an antibody binds with the TCR we expect a response.

2. Inhibitor: When there is an inhibitor, however, it may be possible to block the pathway leading to the activation.

3. Notwithstanding the above, the cell actually has a multiplicity of the previous two and thus there may be some race with a finish line defined by what has been called a "set point", or simply some collection of activators and inhibitors seeing which one dominates.

4. There are not just one possible activator and inhibitor. For a T cell we have the TCR but we may have well more than a PD-1. New inhibitors are arising each day.

5. The internal machinations of the cellular pathways may also effect the net result. Thus, genetic changes can affect what happens.

6. The kinetics of the binding can and often do play a significant role. Binding is not a one-way street, and the result may be loss of tumor control.

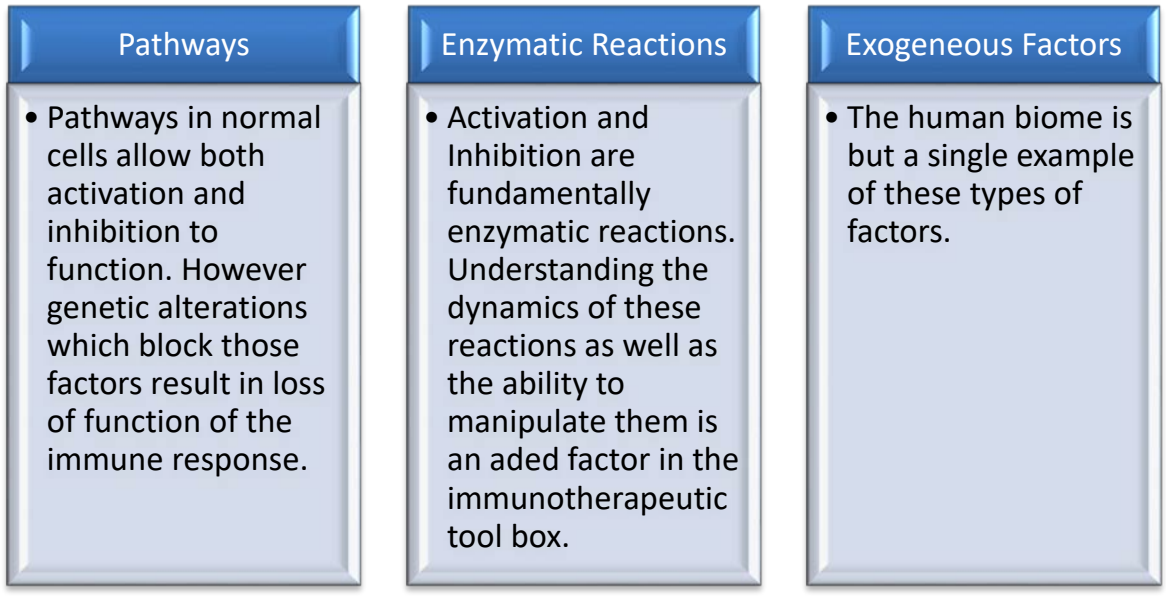
7. Exogeneous Factors: The human biome is often a driving factor to the efficacy of immunotherapy. As Chen and Mellman note:

Factors that are extrinsic to the tumour or host genomes may also affect the immune profile of tumors. Chief among these is the gut microbiome, which has an important role not only in influencing the initiation of some cancers, but also in the response to chemotherapy and immunotherapy…mice bearing subcutaneous syngeneic tumors do not respond to chemotherapy if sterilized by prior treatment with antibiotics or when raised in germ-free conditions. The effect was attributed to the ability of commensal bacteria to activate the innate immune system of the host following chemotherapy, possibly by causing symbiosis and penetration of commensal bacteria into the gut lamina propria.

Subsequent work established an even clearer link between T-cell responses and an intact microbiota1. Fecal transfer or co-housing experiments in mice demonstrated that defined species of gut bacteria enabled antitumor responses after treatment with anti-PD-L1/PD-1 or anti-CTLA4 therapies. Furthermore, the gut microbiota even influenced spontaneous antitumor responses, which correlated with the degree of T-cell infiltration into tumors before any therapy had been administered.

Each of these elements can be considered as a therapeutic target for immunotherapy. We summarize some of these below.

Activator	Inhibitot	Multiplicity	Checkpoints
•Activators start the process of making the immune system attack the putative pathogen	•Inhibitors are often a "self" determinator yet are used by cancer cells to neutralize the immune system.	•The receptors are spread all across the surface of a cell. Also there is a multiplicity of different receptors. Thus, multiplicity has two meanings: • (i) multiplicity of a single type on a single cell, and • (ii) a multiplicity of different types on a single cell. •This is both a complexity and an opportunuity for immunotherapeutic targets.	•Checkpoints are an extension of inhibitors and hev become a key factor in current immunotherapy.



Thus, understanding the specifics may be a useful approach in guiding therapeutic development using the immune system.

3 KILLER CELLS

There are three classes of "killer" cells, each somewhat distinct and providing different approaches to killing pathogens. There are: (i) Cytotoxic T Cells (or Killer T cells or CTL), Natural Killer Cells (or NK cells) and (iii) Natural Killer T cells, NK-T cells. Each is different from the other and each has been used in a variety of ways in treating cancers[1]. I present some fundamental issues on each for coherency in our analysis. This is not a comprehensive overview but it focuses on the key points related to our overall argument

3.1 CTL OF KILLER T CELLS

CTL or Killer T Cells have MHC-I molecules and CD-8 surface proteins. They can be activated through the adaptive immune system. Activation is via IL-2 increase via T Cell helpers. CTLs can bind to a target cell a d they then can conjugate which allows for granule exocytosis which kills the target and then also the CTL to progress to other targets. There are two pathways by which this attack can take; Fas pathway approach and the performin-ganzyme approach.

As Steer et al note:

Although anti-cancer immunity involves both the innate and adaptive immune systems, it is generally held that CD8þ cytotoxic T lymphocytes (CTL) are the most potent anti-tumour effector cell. The T-cell immune response can be broken down into the following steps, all of which need to be fulfilled for effective anti-tumour CTL to be generated:

(1) tumour antigen(s) must be present, and

(2) these must be presented in a context which is seen as dangerous by the immune system;

(3) antigens must be acquired and presented by antigen presenting cells (APC) in the draining lymph node;

(4) specific T cells must then recognize and respond to tumour antigen by proliferating, exiting the lymph node, recirculating and entering the tumour as CTL and

(5) once within the tumour they need to overcome the local immunosuppressive environment before they can kill tumour cells.

In addition, memory cells may need to be generated to produce a sustained response. It is clear that a growing tumour has managed to escape this process. Failure of the anti-tumour immune response can occur at one or more of these steps. Targeting rate limiting steps with therapies designed to boost the immune response can improve anti-tumour immunity.

[1] See Kindt et al, Kuby Immunology, Freeman (New York) 2007; pp 353-368.

In addition to specifically targeted immune therapies, it is also now clear that many traditional cancer therapies can improve key aspects of anti-cancer immunity by inducing tumour cell death in a way that is immunostimulatory or by modulating tumour induced immunosuppression.

3.2 NK CELLS

NK cells are not normal T cells and they deviate from the T cell line earlier in the developmental process. They account for between 5% to 10% of the circulating lymphocytes. They work by producing cytokines and are generally considered a part of the innate immune system.

NK cells have both activation and inhibition receptors. They act in such a manner as to becoming active or inactive by a balancing of activation, it is a thresholding effect.

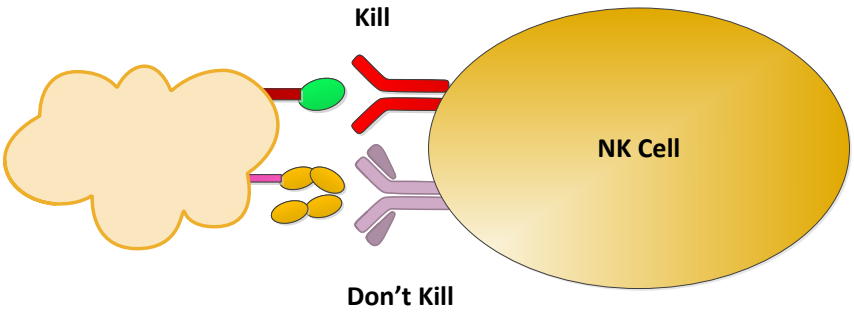
NK cells can be signaled by Interferons, TNF, IL-12 and IL-15. These have been used by researchers in attempts to activate the NK system.

The receptors are lechtin like or immunoglobulin like. The lechtin receptors bind proteins and not lechtins. The second receptors bind HLA-B and HLA-C.

There are inhibitory receptors which are immunoglobulin like such as ILT/LIR as well as KIR, called killer inhibitory receptors.

NK cells have activating and inhibiting ligands. Thus an MHC-I represent a cell which is self and thus has an inhibitory reaction. A second receptor may reflect a viral infection and thus may activate. The actual activation is a balance between inhibition and activation. If the activation is strong enough then even though there may be a inhibitory self-recognition it may be overcome by the activating ligand. This may be a pathway for cancer management. This mechanism is common and is a key point in the discussion of the set points focused on herein.

NK recognizes a marker on the surface and it decides to "kill" the cell. But it also recognizes the MHC I market as self and then does NOT Kill the cell. The MHC I acts as an inhibitor.



What should be noted is the general simplicity of this model. One must remember that there are a multiplicity of these receptors and not just one, that the receptors to function must deal with

internal pathways and that there are a competing set of different receptors. The process is just not as simple as portrayed. This is why on the one hand the Chen and Mellman model has interest.

Now a more detailed discussion by Caligiuri notes:

Years ago, the histologic and functional definition of an NK cell was that of a large granular lymphocyte that could kill a target cell "naturally," that is, in a spontaneous fashion that did not require any priming and was not restricted by the target cell's expression of major histocompatibility complex (MHC) molecules. Experiments in mouse models of bone marrow graft rejection led to the proposal that NK cells would kill any target that lacked self–major histocompatibility complex (MHC) class I molecules (the "missing self" hypothesis).8 This extraordinary idea was developed before anyone knew what the NK cell was using to "see" its targets.

It is now clear that NK cells have a multitude of inhibitory and activating receptors that engage MHC class I molecules, MHC class I–like molecules, and molecules unrelated to MHC. Thus, NK cells are indeed restricted in what target cells they can engage by the expression of the target's MHC ligands, but in a very complex fashion that remains incompletely understood. Notably, orthologs of more recently discovered NK-cell receptor families cannot be found beyond mammals, suggesting that the composite modern day NK cell emerged well after T and B cells appeared to define the vertebrate adaptive immune system.

Furthermore, the complementary roles that NK and cytolytic T cells have in target recognition and host defense, and their similar mechanisms of cytolysis, suggest that these 2 cell types may have each evolved from a common ancestral cytolytic effector cell. Finally, a subset of human NK cells produce abundant cytokines with modest or no ability to lyse target cells. Thus, the older idea of an NK cell as an ancestral forerunner or as a cell defined by a simple function no longer applies. The traditional cell surface phenotype defining human NK cells within the lymphocyte gate on the flow cytometric analyzer shows an absence of CD3 (thereby excluding T cells) and expression of CD56, the 140-kDa isoform of neural cell adhesion molecule (NCAM) found on NK cells and a minority of T cells.

He then goes on to describe what they do in some detail:

Thus, far it has been fully appreciated that NK cells can secrete cytokines and chemokines that influence the host's immune response, and/or kill certain infected or transformed cells via perforin/granzyme or death receptor (e.g., Fas, TRAIL)–related pathways.

Interferon gamma (IFN-) is considered the prototypic NK-cell cytokine, and its production by NK cells is known to shape the Th1 immune response, activate APCs to further up-regulate MHC class I expression, activate macrophage killing of obligate intracellular pathogens, and have antiproliferative effects on viral- and malignant-transformed cells. For many of these functions, it would make sense for NK cells to be in close proximity to APCs and T cells.

Indeed, the subset of NK cells that is the most potent producer of IFN- (i.e., CD56bright NK) is primarily located in the parafollicular T cell– and APC-rich region of SLT.21

As Pittari et al note:

The function of NK cells is governed by a set of germline- encoded activating or inhibitory receptors referred to as killer immunoglobulin-like receptors (KIRs). The extracellular domain determines which HLA class I molecule NK cells recognize, whereas the intracytoplasmic domain transmits either an activating or an inhibitory signal. KIRs are monomeric receptors with either 2 (KIR2D) or 3 (KIR3D) immunoglobulin-like domains, and are further subdivided into those with long (L) cytoplasmic tails (KIR2DL and KIR3DL) and short (S) cytoplasmic tails (KIR2DS and KIR3DS). Long-tail KIRs generate an inhibitory signal through the recruitment of the SH2-domain- containing tyrosine phosphatase 1 protein (SHP1). Short- tail KIRs possess truncated portions that transduce activating signals via tyrosine phosphatase of DAP12 and other proteins.

The NK receptors are also a key element for potential immunotherapy. The KIR receptors are especially the case.

As Vivier et al note:

NK cells were originally described as cytolytic effector lymphocytes, which, unlike cytotoxic T cells, can directly induce the death of tumor cells and virus-infected cells in the absence of specific immunization; hence their name.

Subsequently, NK cells have been recognized as major producers of cytokines such as interferon-g (IFN-g) in many physiological and pathological conditions.

NK cells also produce an array of other cytokines, both proinflammatory and immunosuppressive, such as tumor necrosis factor–a (TNF-a) and interleukin (IL)–10, respectively, and growth factors such as GM-CSF (granulocyte macrophage colony-stimulating factor), G-CSF (granulocyte colony stimulating factor), and IL-3. NK cells also secrete many chemokines, including CCL2 (MCP-1), CCL3 (MIP1-a), CCL4 (MIP1-b), CCL5 (RANTES), XCL1 (lymphotactin), and CXCL8 (IL-8).

Whereas the biological function of the growth factors secreted by NK cells remains to be clarified, their secretion of chemokines is key to their colocalization with other hematopoietic cells such as dendritic cells (DC) in areas of inflammation. Furthermore, the production of IFN-g by NK cells helps to shape T cell responses in lymph nodes, possibly by a direct interaction between naïve T cells and NK cells migrating to secondary lymphoid compartments from inflamed peripheral tissues and by an indirect effect on DC.

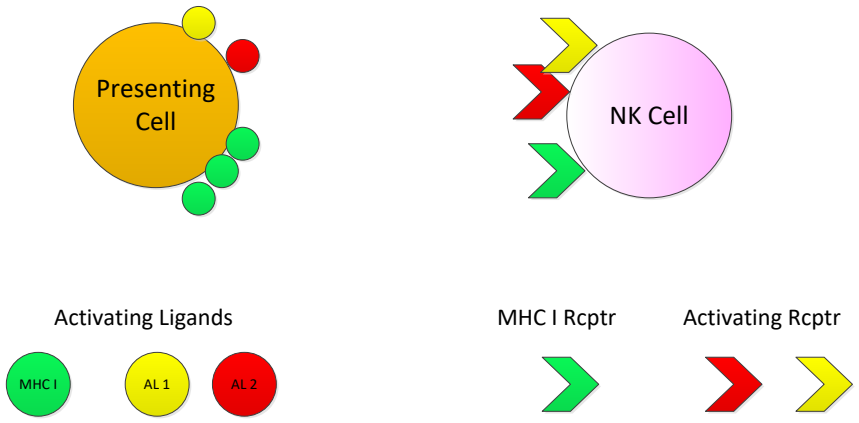
NK cell–mediated killing of target cells also impacts T cell responses, possibly by decreasing the antigenic load and/or because target cell debris might promote antigen cross-presentation to CD8+ cytotoxic T cells. Although NK cells can positively or negatively influence host T and B cell immunity, depending on the nature of the antigenic challenge, the emerging notion is that NK cells are not only cytolytic effector cells against microbeinfected cells or tumor cells. Rather, NK cell– mediated cytotoxicity and cytokine production impact DC, macrophages, and

neutrophils and endow NK cells with regulatory function affecting subsequent antigen-specific T and B cell responses.

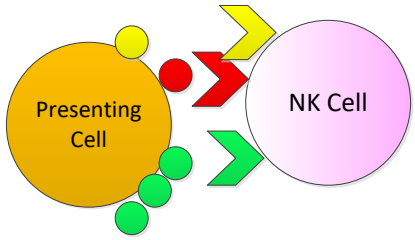
Conversely, the "natural" effector function of NK cells has been revisited. NK cells require priming by various factors, such as IL- 15 presented by DC or macrophages, IL-12 or IL-18 , to achieve their full effector potential, highlighting the intimate regulatory interactions between NK cells and other components of the immune response.

Thus, NK cells, like T and B cells, participate in the immunity in many different ways and undergo a process of functional maturation to fulfill these functions.

Now Vivier et al have described the rather interesting manner in which NK calls can be activated or inhibited which in a sense presages the work of Chen and Mellman. Simply, it is a bit of majority voting by ligands and receptors. We demonstrate this below. Activating ligands can attach to receptors as equally as inactivating.



Then below we demonstrate a somewhat simple majority voting scheme whereby the combination, subject to some putative weighting, can effect either activation or inactivation.



NK is activated if:

Number Activating Ligands > Lmax
and
Number MHC I Ligands < Mmin

else

Not activated

The problem with the above is that we really do not know the threshold. Furthermore we fundamentally do not understand the complexity of the decision making process inside one of these cells. Frankly that will be the challenge in pursuing this area.

Following Vivier et al we note:

NK cells are equipped with an array of receptors that can either stimulate NK cell reactivity (activating receptors) or dampen NK cell reactivity (inhibitory receptors). Activating receptors include receptors that interact with soluble ligands such as cytokines and receptors that interact with cell surface molecules.

Cytokine receptors that are coupled to the common gamma chain (gc), such as IL-15R, IL-2R, and IL-21R, are involved in NK cell development and effector function. In particular, IL-15 is required for the maturation and survival of NK cells, consistent with the absence of circulating NK cells in SCIDX1 patients and in mice lacking IL-15 or IL-15R components. Cytokine receptors that are linked to the adapter protein MyD88 are also important for NK cell maturation, namely IL-1R in humans and IL-18R in the mouse.

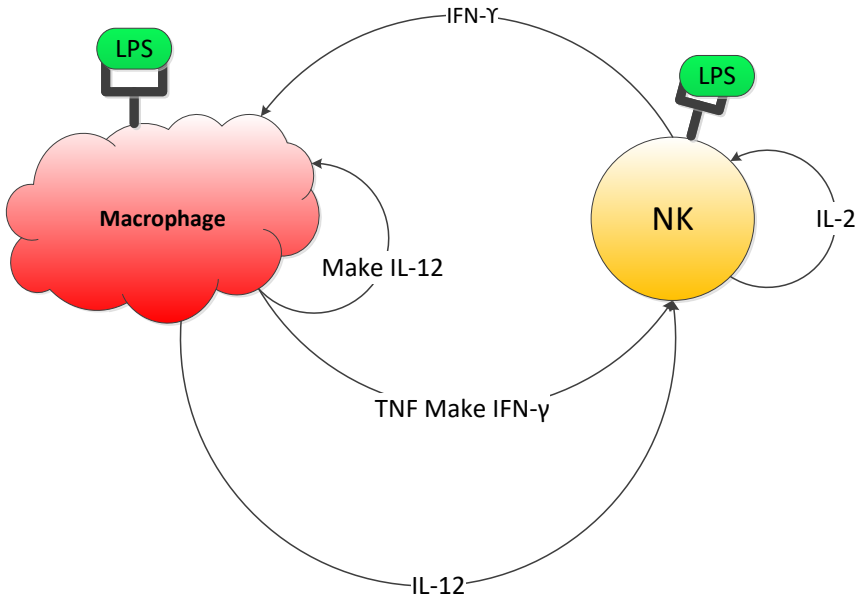
NK cells exert their biological functions by various means. NK cells can kill a variety of target cells, including virus-infected cells and tumors, in the absence of antibody. In the case of viruses, the mouse Ly49H activating receptor recognizes a cytomegalovirus-encoded ligand (m157) (23, 24), and NKp46 has been reported to interact with hemagglutinins derived from influenza and parainfluenza viruses (25).

NK cells are also able to detect antibody-coated cells through the FcgRIIIA (CD16) cell surface receptor and to exert antibody-dependent cell cytotoxicity (ADCC) and cytokine production.

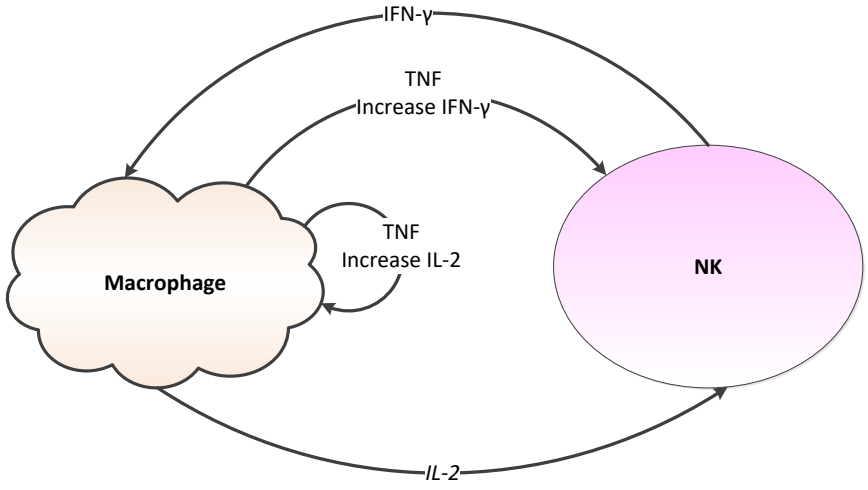
CD16 is coupled to the CD3z and FcRg signal transduction polypeptides bearing intracytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs).

The natural cytotoxicity receptors (NKp46/NCR1, NKp44/NCR2, and NKp30/ NCR3) are also potent activation receptors linked to the ITAMbearing CD3z, FcRg, or DAP12 molecules. In mice, the NK1.1 (Nkrp1c) molecule on CD3– cells has been a useful marker for NK cells, but its expression is confined to only certain strains of mice. NKp46 appears to be the most specific NK cell marker across mammalian species, although discrete subsets of T cells also express it

Now shown below we depict the result of this activation process. There is a flow of Interferons further activating the NK and with the macrophage introduction of a pathogen identifier, in this case a lipo-poly saccharide, LPS, we see the NK then activated and beginning its response.



The figure below is another depiction of this process.

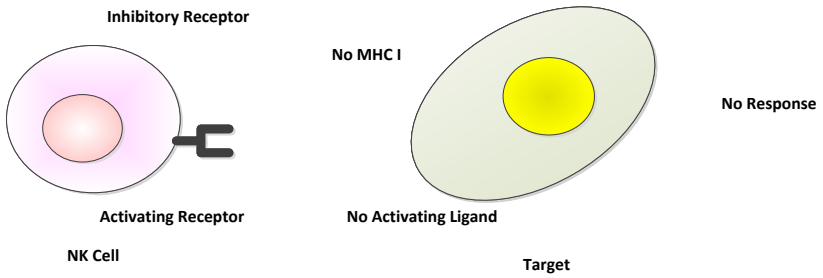


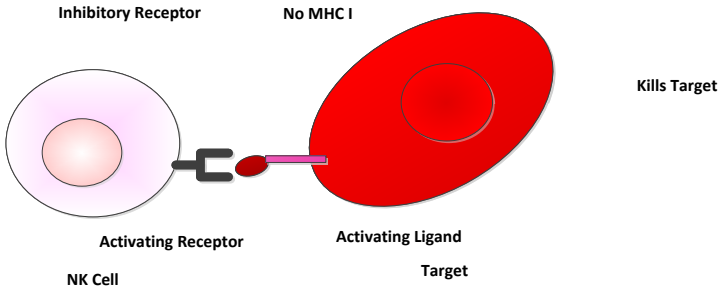
Vivier et al summarizes the various NK cell receptors by function.

Function/ Receptor	Activating Receptors	Inhibitory	Cytokine Receptors	Adhesion Receptors	Activating Adaptors
	NKp46	h KIR-L	IL-1R	CD2	CD3ζ, FcRγ
	CD16	h LILRB1	IL-2R	DNAM-1	CD3ζ, FcRγ
	h NKp30	CD94/NKG2A	IL-12R	β1 integrins	CD3ζ, FcRγ
	h NKp44	m Inh. Ly49	IL-15R	β2 integrins	DAP12
	h NKp80	m NKR-P1B	IL-18R		FcRγ
	m NKR-P1C	m NKR-P1D	IL-21R		DAP10
	NKG2D	KLRG-1	IFNAR		DAP12
	m NKG2D-S	TIGIT			DAP12
	h KIR-S	CEACAM-1			DAP12, DAP10
	m Act. Ly49	LAIR-1			DAP12
	CD94/NKG2C				SAP, EAT2
	CRACC				SAP
	Ly9				SAP, EAT2
	CD84				SAP
	NTBA				SAP, EAT2,
	2B4				ERT

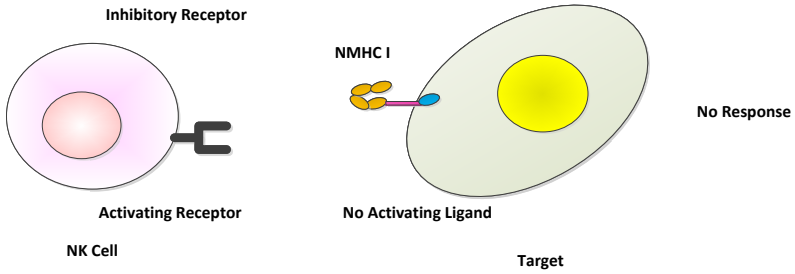
This is clearly a complex set of receptors which serve a multiplicity of functions. Vivier et al also discuss the question of NK being adaptive as well as innate. NK cells are quite powerful and have become cells of interest in a variety of cancer immunotherapeutic applications as we shall show latter.

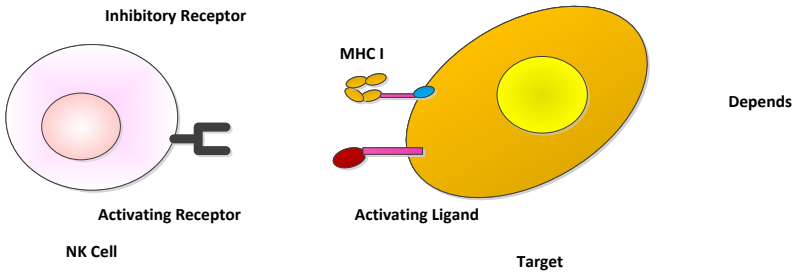
The following graphically demonstrate some of these options:





Then below are the other two options.





These four can be summarized in the Table below.

	Target Activating On	Target Activating OFF
Target Inhibitory On	Depends on Balance	NK Attacks
Target Inhibiting Off	No Response	No Response

Now in our current discussion this simplistic Table is for one Activator and One Inhibitor. The question surrounding the work of Chen and Mellman is:

How do we construct a model wherein the response is a complexity of:

1. Multiple Numbers of the same Inhibitors and/or activators
2. Multiple different Inhibitors and Activators
3. Multiplicity of pathway alterations resulting in variations of resulting responses.

3.3 NK T CELLS

The NK T cell is neither a CTL nor an NK cell. It is a third variety somewhat in between. CTL are adaptive and NK are innate. The T cell receptor on NKT cells does not recognize MHC molecules and it has markers similar to both NK and CTL.

As Ibarrondo et al note:

Invariant natural killer T cells (Type I NKT cells or iNKT) are a subset of T cells that express a restricted repertoire of T-cell receptors (TCR); in humans the iNKT TCR alpha chain presents a Va24-JaQ rearrangement that preferentially pairs with a semiinvariant Vb11 b-chain. The iNKT TCR recognizes glycolipid antigens presented by CD1d, a major histocompatibility complexlike molecule present on the surface of antigen-presenting cells, and that is highly expressed by myeloid dendritic cells (mDCs). iNKT cells are actively recruited to infection sites, where they respond to cytokines and interact with CD1d + mDC. In response to stimuli, iNKT cells can release large amounts of regulatory cytokines and are believed to play a pivotal role in the determination of innate and adaptive immune system responses.

iNKT cells can be subdivided into three subsets: CD4 + , CD8 + and CD42/CD82 double negative (DN). The CD4 + subset has a Th0 profile, being able to produce Th2 and Th1 cytokines such as interleukin 4 (IL-4) and interferon gamma (IFN-c). DN iNKT cells produce large amounts of Th1 cytokines such as INF-c and tumor necrosis factor alpha (TNF-a), up-regulate perforin, and release low levels of Th2 cytokines in response to stimuli [7]. Finally, CD8 + iNKT cells constitute a Th1-only subse.

The balance of CD4 + versus DN and/or iNKT CD8 + iNKT cells is thought to be critical for proper modulation of immune responses to control inflammatory processes, auto-immunity, and immune surveillance of cancer. The pivotal role of iNKT cells in the regulation of the immune response makes them an attractive target for immunotherapy: the frequency and functionality of iNKT cells is frequently altered in patients with malignancies, autoimmune disorders, and viral infections. Blood iNKT cell frequencies fall in melanoma

As Stetson et al note:

Natural killer (NK) and NK T cells are tissue lymphocytes that secrete cytokines rapidly upon stimulation. Here, we show that these cells maintain distinct patterns of constitutive cytokine mRNAs.

Unlike conventional T cells, NK T cells activate interleukin (IL)-4 and interferon (IFN)- transcription during thymic development and populate the periphery with both cytokine loci previously modified by histone acetylation.

Similarly, NK cells transcribe and modify the IFN- gene, but not IL-4, during developmental maturation in the bone marrow. Lineage specific patterns of cytokine transcripts predate infection and suggest evolutionary selection for invariant but distinct types of effector responses among the earliest responding lymphocytes. NK cells are required for effective host defense against herpes viruses in mice and humans.

Although the precise evolutionary niche subserved by NK T cells is not completely clear, the capacity of NK T cells to activate rapid cytokine expression has been exploited to manipulate the outcomes of autoimmunity and cancer. Aside from their expression of common NK-associated surface antigens, such as NK1.1, NK T and NK cells share developmental requirements. Deficiencies in certain cytokines, such as IL-15 or lymphotoxin, or transcription factors such as Ets-1 or Irf-1, lead to loss of both cell lineages. Recent studies suggest their capacity to express cytokines rapidly may also be developmentally acquired .

Although other studies elegantly demonstrate how these cells become activated , the mechanisms underlying their rapid cytokine production or their distinct cytokine patterns, IFN- in the case of NK cells and both IL-4 and IFN- in the case of NK T cells, remain unknown. Elucidation of such mechanisms may have important implications for understanding polarized cytokine production by T cells in adaptive immune responses.

We demonstrate that NK T cells and NK cells, distinguished by their ability to mobilize effector cytokines rapidly after immunization or infection, reside in the periphery spontaneously poised with constitutive cytokine transcripts.

Modification of the respective cytokine loci in a manner promoting access by transcription factors correlates with the presence of cytokine mRNAs. Unlike conventional T cells, NK T and NK cells activate transcription of cytokine genes during early development in the thymus and bone marrow, respectively. In the case of IL-4 for NK T cells, neither the percentage of IL-4

4 SOME THEORY?

The previous brief summary lays out some of the issues inherent in the Chen and Mellman paper. To better understand let us now return to the ideas of Chen and Mellman. Specifically their definition of a "set point". They state:

The cancer–immune set point is the threshold that must be overcome to generate effective cancer immunity. The set point can be understood as a balance between the stimulatory factors (Fstim) minus the inhibitory factors (Finhib), which together must be equal to or greater than 1, over the summation of all T-cell antigen receptor (TCR) signals for tumour antigens. The cancer–immune set point is shown here:

$$\int (Fstim) - \int (Finhib) \geq 1 / \Sigma\, n=1,\, y\, (TCRaffinity \times frequency)$$

The set point is defined by the summation of the frequency of peptide–MHC–TCR interactions and TCR signalling in all anticancer CD8+ T-cell clones (mainly, the TCR affinity for the antigen–MHC class I complex) against antigens present in the cancer cells, including neoantigens and cancer-associated antigens, and the endogenous balance of the positive and negative immune regulators that are inherent to each host or patient.

Now just what this means is somewhat open for debate because it is written by a biologist not a physical scientist and definitely not an engineer. Permit me to attempt an interpretation. First let us try to be specific about a definition. Namely some definition of a variable which is measurable.

Let us try to first understand the F terms.

Fstim: This is a stimulatory factor. What is it? One could guess it is some cell with an MHC I presenting some antigen Ag to a T cell receptor TCR. Should we examine cell by cell? Should we look at every possible T cell, namely ones that say are CD 8 T cells, or how about other immune cells. Why not include NK cells as well? Should we look at stem cells only, do we know what they are? Do we then count these for every T cell, for a mass of T cells, for what?

Finhib: We know some of these we believe. There is PD-1 and CTLA-4. They can block the T cell from attacking. We also suspect that there are many others we have yet to find. So let us simplistically assume we can model with the two mentioned. But what are we measuring? Are we measuring a single cell, a collection of cells, the totality of all cells? Are we measuring all stimulatory factors or just a few? Are we measuring all inhibitory factors or just the ones we know? Are we weighting some differently than others or the same?

This if we have two single cells and it has say 50 T cell receptors and 45 PD-1 receptors, then we can have activated say 35 of the TCR and have activate say 22 of the PD-1. Now what happens? Is activation by each TCR the same and can a TCR being activated be inhibited by an activated PD-1 on a one to one basis?

$$\int_{S_0'}^{S_1} F_{Stim}(r,t)drdt - \int_{S_0'}^{S_1} F_{Inhibit}(r,t)drdt > \alpha$$

or

$$\sum_{n=1}^{N}\left[\int_{S_0'}^{S_1} F_{Stimulate}(n;r,t)drdt - \int_{S_0'}^{S_1} F_{Inhibit}(n;r,t)drdt\right] > \beta$$

The above still has no physical meaning. Now let us consider T Cell Affinity. As Nicholich et al state:

Affinity refers to the steady-state association constant between a monovalent receptor and its ligand, in this case a single T-cell receptor (TCR) and peptide–MHC (pMHC) complex. Structural avidity is the steady-state association constant between multiple cell-bound receptors and ligands and is determined by the direct binding affinities of multiple TCRs to their pMHC complexes. Functional avidity depends on the relative kinetics of signalling that translate into measurable biological functions such as proliferation, cytokine production or cytolytic function. APC, antigen-presenting cell.

Now as Hsieh et al note:

TCR affinity: The strength of interaction between the T cell receptor and a single peptide–MHC complex.

As an abstraction that may be fine but as something used in a measurement and equation it is highly deficient.

Now as Daniels et al note:

To estimate the TCR affinity of the ligands comprising the selection boundary, we measured tetramer binding; which correlates with monomeric TCR–pMHC affinities, is performed on live cells and involves the participation of CD8. The binding characteristics of tetramers were determined on pre-selection OT-I double positive thymocytes at 37 uC. The dissociation constant (Kd) was calculated by nonlinear regression analysis and confirmed by homologous competition experiments.

The tetramer binding curves for Q4R7 (weakest negative selector), T4 (border ligand) and Q4H7 (strongest positive selector) overlapped. Their Kd values (Q4R7, 4869.5 nM; T4, 55610.1 nM; Q4H7, 5169.1 nM; n57, P50.455) and their half-lives (t1/2) were not significantly different (Table 1). However, heterologous competition assays showed that Q4R7 was more efficient than Q4H7 at inhibiting the binding of OVA tetramers.

or perhaps they mean something akin to this:

$$\sum_{n=1}^{N}\left[\int_{S_0'}^{S_1} F_{Stimulate}(n;r,t)drdt - \int_{S_0'}^{S_1} F_{Inhibit}(n;r,t)drdt\right] > \beta$$

or

$$\frac{\sum_{n=1}^{N}\left[\int_{S_0'}^{S_1} F_{Stimulate}(n;r,t)drdt - \int_{S_0'}^{S_1} F_{Inhibit}(n;r,t)drdt\right]}{\sum_{m=1}^{M} TCR_{Affinity}(m)\, f(m)} > \lambda$$

Now we know that there is a threshold effect for activating and suppressing. Namely there has to be more activators than suppressors. Just what that balance is of course is uncertain. Again, the statement has no physical meaning.

They continue:

This can be further influenced by other elements of immunity, including tumour-derived immunomodulatory components, as well as by exogenous factors such as infection and exposure to pharmacological agents. A given patient with cancer may have a low set point, making it easier to generate an anticancer immune response, or a high set point, which makes it more difficult.

The aim of immunotherapy is to increase Fstim, decrease Finhib or increase TCR signalling to drive progression of the cancer-immunity cycle. These values are difficult to quantify with current techniques but represent a useful theoretical construct. It is probable that the cancer–immune set point of a particular person is already determined by the time of clinical presentation, driven by the inherent immunogenicity of the tumour and by the responsiveness of the individual's immune system.

Although it is reasonable to assume that various lines of cancer therapy or changes in environmental factors might alter Fstim and Finhib, such changes might only be transient. Often, the set point that is identified using pretreatment biopsies is similar to the set point determined by biomarker profiling from biopsies taken on progression after therapy.

Likewise, despite the continued accumulation of mutations in a tumour as a function of time, primary and metastatic lesions can exhibit similar immune profiles. The features that determine the set point may therefore reflect genetic factors that are specific to a given tumour, the genetics of the person with cancer, or the extent to which antitumor immunity had developed initially. Conceivably, immunotherapy may work as a consequence of either its direct effect on Fstim and Finhib (that is, by assisting the completion of a single revolution of the cancer-immunity cycle) or its ability to alter the set point (for example, by propagating the cancer-immunity cycle, which enhances the cancer-specific T-cell response).

Although largely conceptual, the idea of a set point provides a framework to help organize the torrent of clinical and biomarker data that will emerge over the coming months and years. The number of targets that could prove effective for cancer immunotherapy is great; the number of

potential combinations of therapeutic agents that are directed against these targets (or combinations of such agents with conventional standard-of-care agents) is even greater.

Thus, let us try and construct meaning which may be measurable and verifiable as well as actionable. Consider the following model:

1. Let us assume we have a tumor cell. Let us assume there are N possible activator ligands and M inhibitor ligands.

2. Let us assume that for each of the above ligands we have on a T cell some receptor. If there is a ligand without a receptor we shall ignore it.

3. Assume we can count and differentiate the differing ligand-receptor possibilities on a cell.

4. Now calculate the following:

$$\frac{\sum_{k=1}^{K} \alpha_k N_{activator,k} - \sum_{j=1}^{J} \beta_j N_{Inhibitor,j}}{\sum_{k=1}^{K} N_{activator,k} + \sum_{j=1}^{J} N_{Inhibitor,j}} \leq \lambda$$

Namely, we count the number of different activators and the number of different inhibitors and then weight them by some metric, yet to be determined, and then weigh them by the total present.

This approach may have merit. The weights may be unity, but that is a mere guess. The weights may be reflective of the enzymatic consistency of the contact. Frankly we just do not know but it is worth exploring.

Consider the following model:

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However, if we have to consider the binding dynamics we would have:

$$TCR + pepMHC \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} TCR : pepMHC$$

where TCR is the T Cell receptor and pepMHC is, the antigen presenting particle on the MHC surface molecule complex.

Thus, N as above is a random variable, in fact a random process. That is for any activator or inhibitor at any time:

$$N(t) = \sum_{k=1}^{K} n_k(t)$$

where

$$n_k(t) = \begin{cases} 1; P[n=1] = p \\ 0; P[n=0] = 1 - p = q \end{cases}$$

and

$$E[N(t)] = pK$$

That is each N(t) is a random process where it is characterized by two parameters; the maximum number and the probability of binding. If K were large enough then we could use the central limit theorem to provide a Gaussian distribution with mean and variance. Namely:

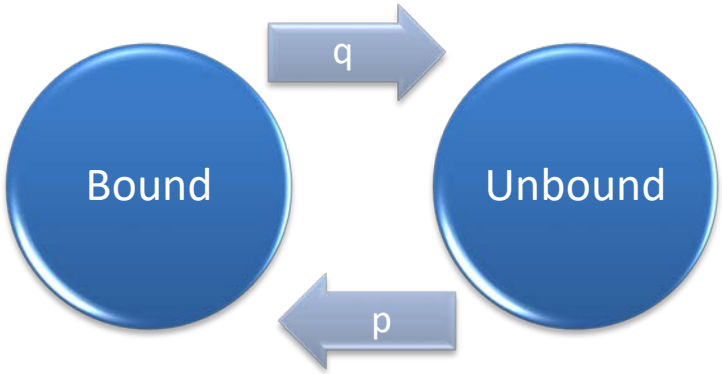
$$p_{n_i}(x) = N(p_i K_i, p_i q_i K_i)$$

where N represent the normal density with mean pK and variance pqK.

Thus, when we examine the statistic above we can write it as:

$$\Lambda(t) = \frac{\Delta(t)}{\Pi(t)} \geq \lambda$$

where numerator and denominator are Gaussian. However, if the number or receptors is large then both numerator and denominator reduce to near constants. On the other hand, if there is but one pair of each we have a random process.



This process is commonly known as the "Telegraph Process"[2]. Namely it is a simple on-off system and as such it would either suppress or activate the process. Depending on how large the inhibition is, or the activation is, will be reflected in the time the cells are controlled by the immune system. From a therapeutic perspective, the question is; is there a mechanism to keep it active at a higher rate?

[2] See Parzen, Stochastic Processes, Holden Day, 1962.

5 T CELL MECHANICS

We briefly will examine the now classic model of Check Points, specifically the PD-1 Check Point which has received a great deal of attention.

From Freeman, we have an excellent summary description:

T cell activation requires a TCR mediated signal, but the strength, course, and duration are directed by costimulatory molecules and cytokines from the antigen-presenting cell (APC).

An unexpected finding was that some molecular pairs attenuate the strength of the TCR signal, a process termed coinhibition.

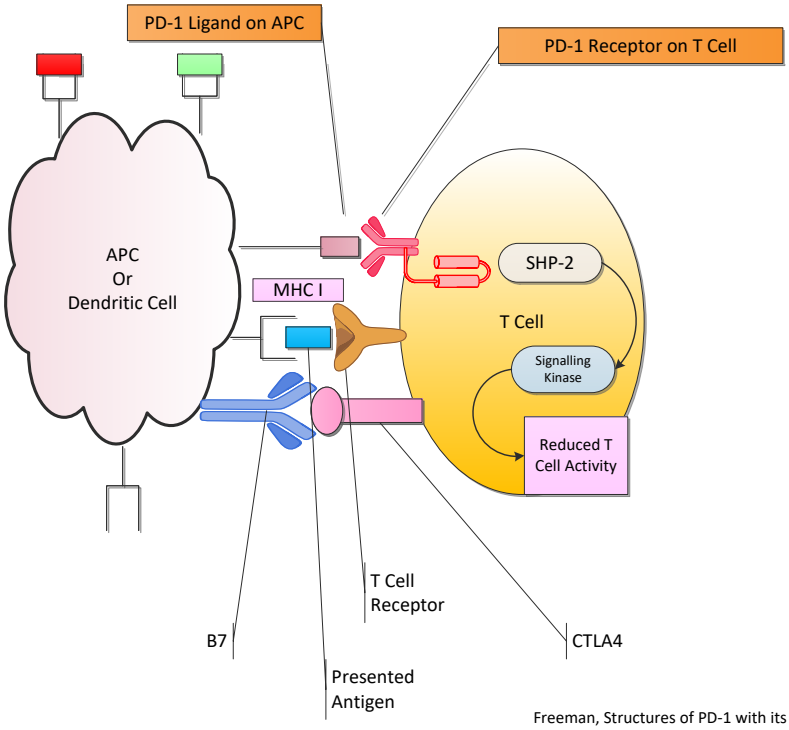
The threshold for the initiation of an immune response is set very high, with a requirement for both antigen recognition and costimulatory signals from innate immune recognition of ‘‘danger’’ signals. Paradoxically, T cell activation also induces expression of coinhibitory receptors such as programmed death-1 (PD-1).

Cytokines produced after T cell activation such as INF- and IL-4 up-regulate PD-1 ligands, establishing a feedback loop that attenuates immune responses and limits the extent of immune-mediated tissue damage unless overridden by strong costimulatory signals. PD-1 is a CD28 family member expressed on activated T cells, B cells, and myeloid cells. In proximity to the TCR signaling complex, PD-1 delivers a coinhibitory signal upon binding to either of its two ligands, PD-L1 or PD-L2.

Engagement of ligand results in tyrosine phosphorylation of the PD-1 cytoplasmic domain and recruitment of phosphatases, particularly SHP2. This results in dephosphorylation of TCR proximal signaling molecules including ZAP70, PKC, and CD3, leading to attenuation of the TCR/CD28 signal.

The role of the PD-1 pathway in peripheral T cell tolerance and its role in immune evasion by tumors and chronic infections make the PD-1 pathway a promising therapeutic target. Two recent papers have determined the structures of the PD-1/PD-L1 and PD-1/PD-L2 complexes. PD-L2 (B7-DC; CD273) is inducibly expressed on dendritic cells and macrophages, whereas PD-L1 (B7-H1; CD274) is broadly expressed on both professional and nonprofessional APCs as well as a wide variety of nonhematopoietic cell types. The PD-1 pathway is important for the maintenance of peripheral T cell tolerance.

This process is shown graphically below. All three elements are shown; activator, inhibitor, and pathway. What is not shown are the multiplicity effects.



Freeman, Structures of PD-1 with its ligands: Sideways and dancing cheek to cheek

Now if we have a simple model as above we then consider for a therapeutic a mechanism for blocking the Check Point. Namely design for example a monoclonal antibody, Mab, which can overpower the PD-1 receptor and inhibit is reaction. This has been the basis for many such therapies.

rom Galluzzi et al we have the following list of Mabs used or in study for various cancers.

Alemtuzumab	**Chronic lympocytic leukemia**	**2001**	**Selective recognition/opsonization of CD52+ neoplastic cells**
Bevacizumab	Colorectal carcinoma Glioblastoma multiforme Cervical carcinoma Lung carcinoma Renal cell carcinoma	2004	VEGFA neutralization
Brentuximab vedotin	Anaplastic large cell lymphoma Hodgkin's lymphoma	2011	Selective delivery of MMAE to CD30+ neoplastic cells
Blinatumumab	Acute lymphoblastic leukemia	2014	CD3- and CD19-specific BiTE
Catumaxomab	Malignant ascites in patients with EPCAM+ cancer	2009	CD3- and EPCAM-specific BiTE
Ipilimumab	Melanoma	2011	Blockage of CTLA4-dependent immunological checkpoints
Nivolumab	Melanoma	2014	Blockage of PDCD1-dependent immunological checkpoints
Pembrolizumab	Melanoma	2014	Blockage of PDCD1-dependent immunological checkpoints
Cetuximab	Head and neck cancer Colorectal carcinoma	2004	Inhibition of EGFR signaling
Denosumab	Breast carcinoma Prostate carcinoma Bone giant cell tumors	2011	Inhibition of RANKL signaling
Gemtuzumab ozogamicin	Acute myeloid leukemia	2000	Selective delivery of calicheamicin to CD33+ neoplastic cells
Ibritumomab tiuxetan	Non-Hodgkin lymphoma	2002	Selective delivery of 90Y or 111In to CD20+ neoplastic cells
Panitumumab	Colorectal carcinoma	2006	Inhibition of EGFR signaling
Pertuzumab	Breast carcinoma	2012	Inhibition of HER2 signaling
Obinutuzumab	Chronic lymphocytic leukemia	2013	Selective recognition/opsonization of CD20+ neoplastic cells
Ofatumumab	Chronic lymphocytic leukemia	2009	Selective recognition/opsonization of CD20+ neoplastic cells
Ramucirumab	Gastric or gastroesophageal junction adenocarcinoma	2014	Inhibition of KDR signaling
Rituximab	Chronic lymphocytic leukemia Non-Hodgkin lymphoma	1997	Selective recognition/opsonization of CD20+ neoplastic cells
Siltuximab	Multicentric Castleman's disease	2014	IL-6 neutralization
Tositumomab	Non-Hodgkin lymphoma	2003	Selective recognition/opsonization of, or selective delivery of 90Y or 111In to, CD20+ neoplastic cells
Trastuzumab	Breast carcinoma Gastric or gastroesophageal junction adenocarcinoma	1998	Selective recognition/opsonization of, or selective delivery of mertansine to, HER2+ cancer cells

The interesting observation regarding Mabs is that they require some check point type inhibitor plus they must not cause massive check point failures elsewhere. One should always be concerned

with what can be called the "carpet bombing" effect. Namely in targeting one aberrant cell we manage to kill an excessive number of bystanders to the detriment of the patient.

6 BACK TO T CELL IMMUNE SET POINTS

We now return to following Chen and Mellman and their observations. They note:

The role of the immune system in cancer remained unappreciated for many decades because tumors effectively suppress immune responses by activating negative regulatory pathways (also called checkpoints) that are associated with immune homeostasis or by adopting features that enable them to actively escape detection.

Two such checkpoints, cytotoxic T-lymphocyte protein 4 (CTLA4) and programmed cell death protein 1 (PD-1), have garnered the most attention so far.

CTLA4 is a negative regulator of T cells that acts to control T-cell activation by competing with the co-stimulatory molecule CD28 for binding to shared ligands CD80 (also known as B7.1) and CD86 (also known as B7.2).

The cell-surface receptor PD-1 is expressed by T cells on activation during priming or expansion and binds to one of two ligands, PD-L1 and PD-L2. Many types of cells can express PD-L1, including tumour cells and immune cells after exposure to cytokines such as interferon (IFN)-γ; however, PD-L2 is expressed mainly on dendritic cells in normal tissues. Binding of PD-L1 or PD-L2 to PD-1 generates an inhibitory signal that attenuates the activity of T cells. The 'exhaustion' of effector T cells was identified through studies of chronic viral infection in mice in which the PD-L1/PD-1 axis was found to be an important negative feedback loop that ensures immune homeostasis; it is also an important axis for restricting tumour immunity.

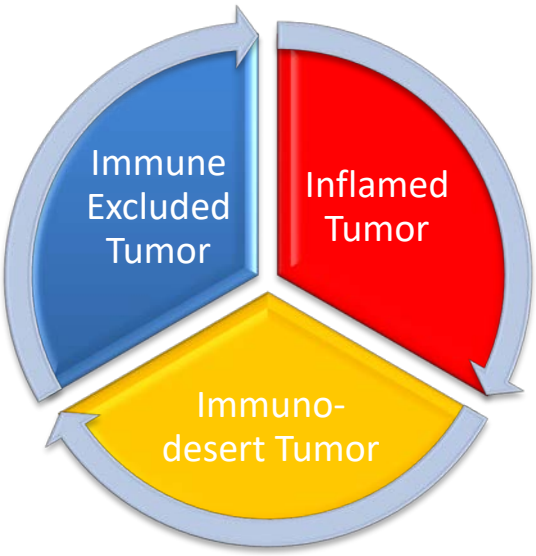
They then proceed to characterize three differing states of tumors with respect to their T cell response. They are:

1. Inflamed Tumor: This a tumor with lots of cells and penetrating the tumor space.

2. Immune Desert Tumor: This is a tumor with lots of cells but no significant penetration of the tumor space.

3. Immune Excluded Tumor: This is a tumor with a paucity of any T cells present.

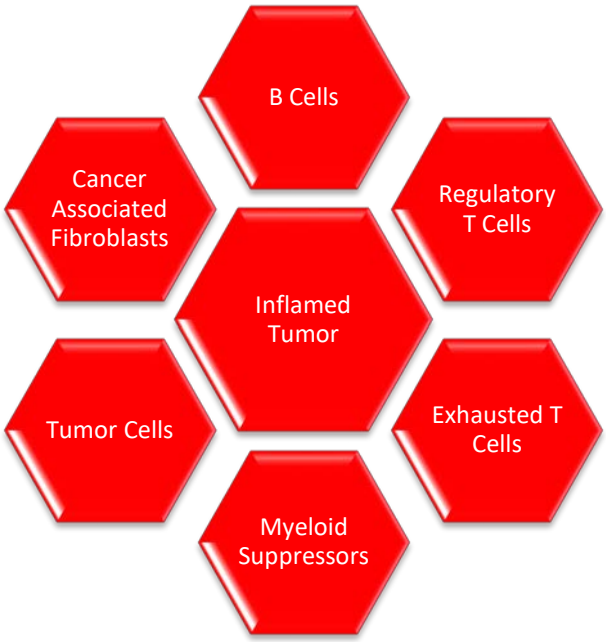
We depict those three types below.



Now we consider the descriptions as presented by Chen and Mellman:

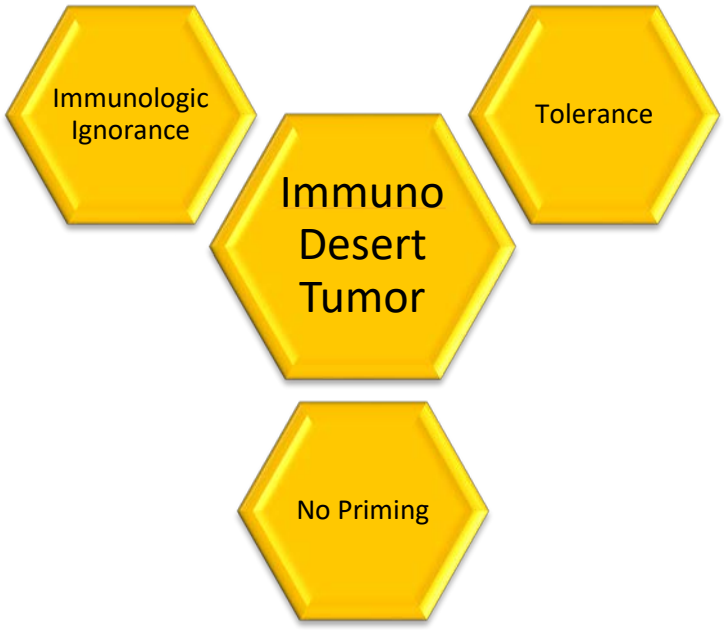
The first profile, the immune-inflamed phenotype, is characterized by the presence in the tumour parenchyma of both CD4- and CD8-expressing T cells, often accompanied by myeloid cells and monocytic cells; the immune cells are positioned in proximity to the tumour cells. Samples from inflamed tumors may exhibit staining for PD-L1 on infiltrating immune cells and, in some cases, tumour cells. Many proinflammatory and effector cytokines can also be detected by mRNA analysis in these sections of tumors. This profile suggests the presence of a pre-existing antitumor immune response that was arrested probably by immunosuppression in the tumour bed. Indeed, clinical responses to anti-PD-L1/PD-1 therapy occur most often in patients with inflamed tumors…

We depict the characteristics graphically below:



The second profile is the immune-excluded phenotype, which is also characterized by the presence of abundant immune cells. However, the immune cells do not penetrate the parenchyma of these tumors but instead are retained in the stroma that surrounds nests of tumour cells. The stroma may be limited to the tumour capsule or might penetrate the tumour itself, making it seem that the immune cells are actually inside the tumour. After treatment with anti-PD-L1/PD-1 agents, stroma-associated T cells can show evidence of activation and proliferation but not infiltration, and clinical responses are uncommon. These features suggest that a pre-existing antitumor response might have been present but was rendered ineffective by a block in tumour penetration through the stroma or by the retention of immune cells in the stroma. T-cell migration through the tumour stroma is therefore the rate-limiting step in the cancer–immunity cycle for this phenotype.

We depict the characteristics of this class below:



Finally, the third type is characterized as follows:

The third profile, the immune-desert phenotype, is characterized by a paucity of T cells in either the parenchyma or the stroma of the tumour. Although myeloid cells may be present, the general feature of this profile is the presence of a non-inflamed tumour microenvironment with few or no CD8-carrying T cells. Unsurprisingly, such tumors rarely respond to anti-PD-L1/PD-1 therapy. This phenotype probably reflects the absence of pre-existing antitumor immunity, which suggests that the generation of tumour-specific T cells is the rate-limiting step. The immune-desert phenotype and the immune-excluded phenotype can both be considered as non-inflamed tumors.

Thus, this does pose the question; how does one identify these cells and how could one move one category to the other for better response? Frankly one asks just what is happening from one class to another?

7 OBSERVATIONS

Set Points, Check Points, and other elements of the control of the immune system as a mechanism to understand and deal with cancer has been evolving at a rapid pace. Where the Check Point field seeks new and effective ligand-receptor pairs, the Set Point field seems to examine the process in a more holistic manner. Perhaps that is an approach which would enable a more systematic approach.

7.1 CELL MATURATION AND DIFFERENTIATION

How does this process change as a cell matures? What of cell differentiation. T cells like many of the lymphoid line go through varying degrees of maturation. Thus, we ask: what is the difference?

7.2 STEM CELLS

We have discussed the stem cell constructs at length. In McGarty (Stem Cells) we have tried to bring some of these ideas up to data. The problem is that stem cells may very well have different markers than the cells we can attack with the tools at hand. Thus, attacking PD-1 and CTLA4 markers may work for the mass of the tumor and result in shrinkage but it may totally miss the stem cell. How best to address this is uncertain?

7.3 THERAPEUTIC DIMENSIONS

What are the therapeutic dimensions of this principle? We have discussed a few here but there are many which present themselves.

7.4 CAR T CELLS

CAR-T cells are "engineered" T cells which are designed by use of such tools as a lentivirus to attack a specific malignant cell. They have been shown to be useful for hematological cancers and have been examined for solid tumors. As Ramachandran notes in his Thesis:

As the name suggests, a CAR is a chimera of domains from different proteins assembled together to create a functional receptor. These novel receptors initiate a functional downstream effector T-cell signaling pathway when they encounter target antigen, usually the TAA on a cancer cell. This gives the opportunity to engineer a large variety of TAA-specific receptors targeting a broad range of cancer types.

CARs typically contain four domains

(a) extracellular antigen binding domain: It confers the antigen-specificity to the engineered T-cell. A majority of the engineered CARs for cancer therapy have antibody-derived antigen binding domains called single-chain variable fragment (scFv). CARs containing a scFv extracellular domain retain the specificity of an antibody. A major advantage of having scFv

extracellular domain is that it bypasses the need for antigen presentation by MHC-I on tumor cells, as antibodies directly bind to cell surface antigens.

(b) Spacer or hinge region: It gives flexibility and length to allow proper dimerization of scFv, thus improving its stability. The most commonly used spacer regions are derived from IgG Fc CH2-CH3 domains, CD28 hinge domain and CD8α spacer domain

(c) Transmembrane domain: It determines the stability of CAR expression on cell surface. The most commonly used transmembrane regions are derived from CD3ζ CD4, CD8 and CD28 molecules138 and

(d) Cytoplasmic signaling domain(s): This region has the domains that provide the necessary downstream signaling for T-cell effector functions.

CARs are classified into different generations based on the number of cytoplasmic signaling domains namely first, second and third generation CARs. First generation CARs have only one cytoplasmic domain, usually T-cell activation signaling domain (CD3ζ chain). In addition to the T-cell activation domain second generation CARs have one extra co-stimulatory signaling domain, e.g., CD28, 4-1BB, ICOS or OX40 and third generation CARs have two extra co-stimulatory domains…

In a recent Technical Note McGarty has further developed the CAR-T cell concepts for both hematological and solid tumors. CAR-T are engineered to specific targets. The question then is; can a better understanding of set points allow for improved targeting for CAR-T cells or are CAR-T cells perforce of their design not really useful for attacking solid tumors?

7.5 DYNAMIC MODELS

The enzyme kinetics of the reactions on the surfaces of T cells and APC or tumor cells are critical. We have almost always assumed that once a protein is bound it stays. Yet we know it is not the case. Furthermore, when understanding the set point model, if we have a paucity of activators on a T cell it will not function. If the paucity is due to enzymatic action, then perhaps we can indirectly address the low level by increasing the retention via enzyme kinetic improvement.

7.6 PATHWAY FACTORS

The pathway factors are both integral to immunotherapeutic approaches, they facilitate the process inside a T cell for example, but they may also be poorly understood. Let us briefly review that issue. We must look at pathways from the perspective of the T cell and the tumor Ag presenting cell.

1. From the T cell perspective we have internal genetic pathways which facilitate the process of cytokine release. If there are faults on the pathway, then we would not expect the T cell to function. Thus, we may ask if these are somatic defects or a result of some change in the T cell.

2. From the perspective of the tumor cell, we know its pathways have usually been altered. Then does this altering result in the excess expression of inhibitors or the suppression of activators. Do the pathways alter the MHC I presentation efforts?

Both dimensions are worth examining.

7.7 POLITICAL FACTORS

A recent National Academies Report by Balogh et al present several policy issues regarding immunotherapy. The report was meant to present a simplified overview of immunotherapeutics as well as present some key policy issues. Concerns regarding costs, patient value, physician-patient expectations were discussed. Also was a discussion on evidence based approaches. The problem is that the experience is limited and the costs high. Furthermore what seemed not discussed was the fact that the complexity of this field is great and the depth of understanding by physicians quite limited. One could say that most Oncologists are trained to administer chemotherapy, and have a limited if not aged understanding of the immune system.

8 REFERENCE

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