T cell exhaustion

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Disclosures

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Founder and Scientific Advisor: TTMS, Inc.

Disclosures

I am thrilled to be invited to give a one-hour talk on T cell exhaustion.

However, exhaustion is still a controversial topic, as not everyone necessarily agrees what it means to be exhausted (or if it even *is* its own state/fate)

Thus, talking about it in its entirety can be quite.. political.

I will thus try to give a balanced talk (more CNN, less Fox News) regarding the importance of this functional fate in cancer immunology.

If you are trying to turn on T cells, it's critical that said T cells have the capacity to be functional

- However T cells have a wide variety of functional and differentiation states, and some of them are dysfunctional **even with proper stimulation**
  - **Anergy**: A functional state in which TCR triggering alone induces a transcriptional and post-translational program preventing future reactivity through the TCR (even if that future stimulation *includes* costimulation)
  - **Senescence**: A pathologic state in which time and extensive cell division result in T cells that are chronically secretory but respond poorly to antigen
    - Also lose costimulatory receptors preventing a full stimulation
  - **Exhaustion**: A terminal differentiation state driven by persistent antigen that results in progressive loss of polyfunctionality
- All of these dysfunctional states seem to be 'read out' the same way
  - Loss of cytokine production
  - Loss of proliferative capacity
  - Loss of cytotoxicity
- **All of these dysfunctional states are important in cancer!**

History of T cell exhaustion

TABLE 1

LCMV-specific CTL and Antibody Responses in Acutely and Persistently Infected BALB/c WEHI Mice

CTL response*

TABLE VIII

Suppression of CTL Response and Establishment of Persistent Infection by LCMV Clone 13, a Genetic Variant Isolated from Spleen of Carrier Mice

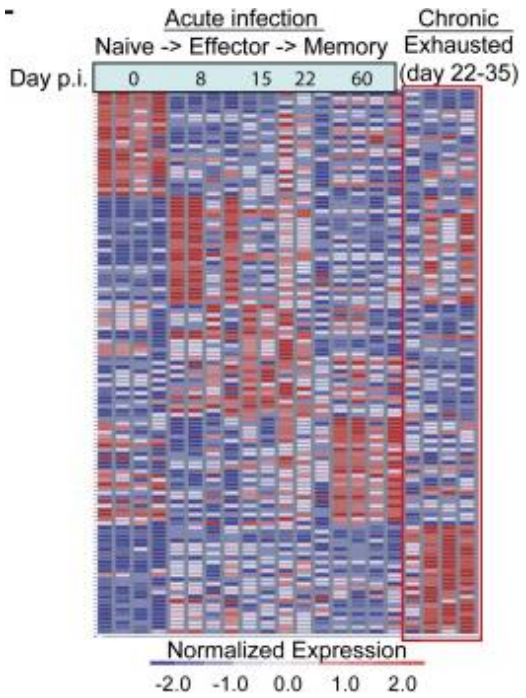
Source of spleen cells transferred*	Challenge virus	LCMV-specific CTL in spleen: Percent specific ^{51}Cr release from BALB CL-7 ($H\text{-}2^d$) targets (E/T, 50:1)			LCMV-specific antibody in serum	LCMV titer in serum (log_{10} PFU/ml)				
						Days postinfection				
		Uninfected	LCMV Armstrong infected	LCMV clone 13 infected	ELISA titer (log 2)	8	30	45	55	90
Normal	LCMV Armstrong	3	58	52	13.9	<1.6	<1.6	<1.6	NT‡	NT
		0	52	54	14.1	<1.6	<1.6	<1.6	NT	NT
		1	52	57	13.7	<1.6	<1.6	<1.6	NT	NT
Normal	LCMV Clone 13§	2	6	8	12.7	4.7	5.4	4.1	4.3	5.1
		0	0	0	14.6	4.3	5.3	4.2	4.6	4.3
		0	2	3	13.3	3.9	5.1	4.7	NT	NT
Normal	LCMV Armstrong + LCMV clone 13	3	7	12	13.3	4.0	5.4	4.8	5.3	4.7
		0	6	2	13.8	4.1	5.4	4.2	5.3	3.9
		1	8	7	12.9	3.8	5.1	5.0	5.3	5.1

* 5×10^7 spleen cells from adult BALB/c WEHI mice were transferred intravenously into normal adult BALB/c WEHI mice

rier mice)	2	0	4	5	2	2	6	<4.7
	3	2	3	3	2	2	4	<4.7
	4	4	2	3	4	5	7	<4.7
	5	5	2	4	2	2	5	9.8
	6	0	1	5	0	1	3	7.5

- The term ‘exhaustion’ was coined thirty years ago in chronic viral infection
- A major advance of the ability to study exhausted T cells was in lymphocytic choriomeningitis virus (LCMV) infection in mice
- LCMV has two commonly used strains that differ by two amino acids
- This amino acid results in antigen chronicity in vivo

Chronic infections helped identify targets present on exhausted T cells



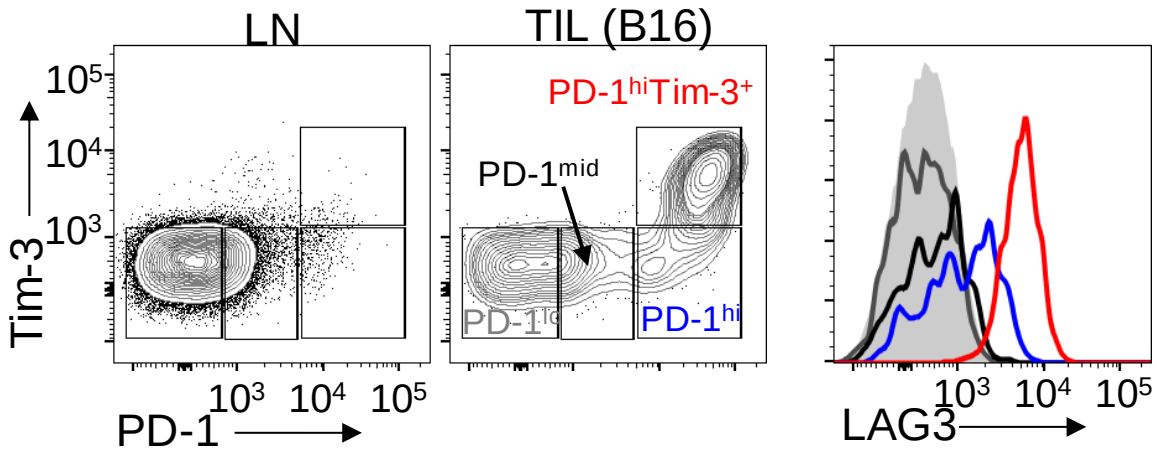
I. Inhibitory receptors				
Gp49b	12.24	4.29	4.48	
Cd244	11.02			
Ctla4	6.93	2.30	2.33	
Pdcd1	6.55			
Cd160	4.08			A
Klrc1		4.41	2.03	A
Ptger4	4.07			
Gp49b	4.04	2.56	2.49	
Lag3	3.25			
Klra9	2.88	2.04		
Klrg1	2.59	36.90	7.36	A
Klra3	2.20			
Ptger2	2.08			A

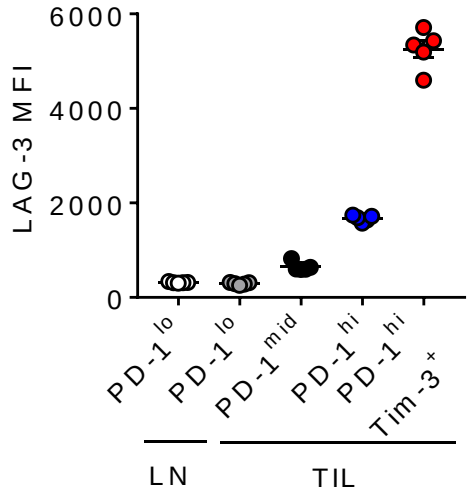
II. Cell surface receptors and ligan				
Tnfsf6	16.29	6.12	5.61	
Ctla2b	8.64	14.44	11.60	
Ctla2b	8.30	12.72	12.77	
Itgax		7.60	3.07	A
Nrp	5.65			
Tnfrsf9	5.42			A
Pglyrp1	5.01			A
Tmem49	4.62	2.79		A
Il18r1		4.21	4.93	
Klrk1		4.05	3.86	A
Adam19	3.80	2.37		A
Itga4		3.75		
Klrk1		3.58	2.56	A
Ly6c		3.56	2.79	
Itgb1	3.52	3.94	3.61	
Tnfrsf1b	3.48			
Ly6a	3.24	4.06	4.07	
Alcam	2.92			
Cd9	2.60			
Pglyrp1	2.50			A
Igsf10		2.48		A
Cd7	2.45			
Glycoprot	2.38			
Itga4	2.35	4.35		A
Tnfrsf1a	2.31			A
Cd48		2.26		
Itgb2		2.26		
Fcgr2b		2.23	2.45	
Il1rl1		2.19		
Itgav	2.18			A
Itgb1	2.17			
Gpr65	2.12			
Cdam			2.11	A

- Microarray analysis of T cells responding to chronic versus acute viral infection revealed several cell surface molecules highly upregulated on T cells as they progressed to exhaustion
  - PD-1
  - LAG3
  - TIM-3/HAVCR2
  - TIGIT
  - 2B4
  - CD160
  - And so on…
- However, costimulatory molecules are also overexpressed on terminally exhausted T cells (4-1BB, OX40, GITR)
- In other words: these cells are **hungry for signals** and thus become dependent on the presence of the ligands: a dangerous proposition in the tumor microenvironment

Observing similar phenotypes in cancer-responsive T cells

- As cancer represents a source of chronic activation, T cells in tumors also succumb to T cell exhaustion
- Consistent with chronic infections, tumor reactive T cells progressively upregulate co-inhibitory molecules and lose polyfunctionality
- However, this is still a hotly debated topic!



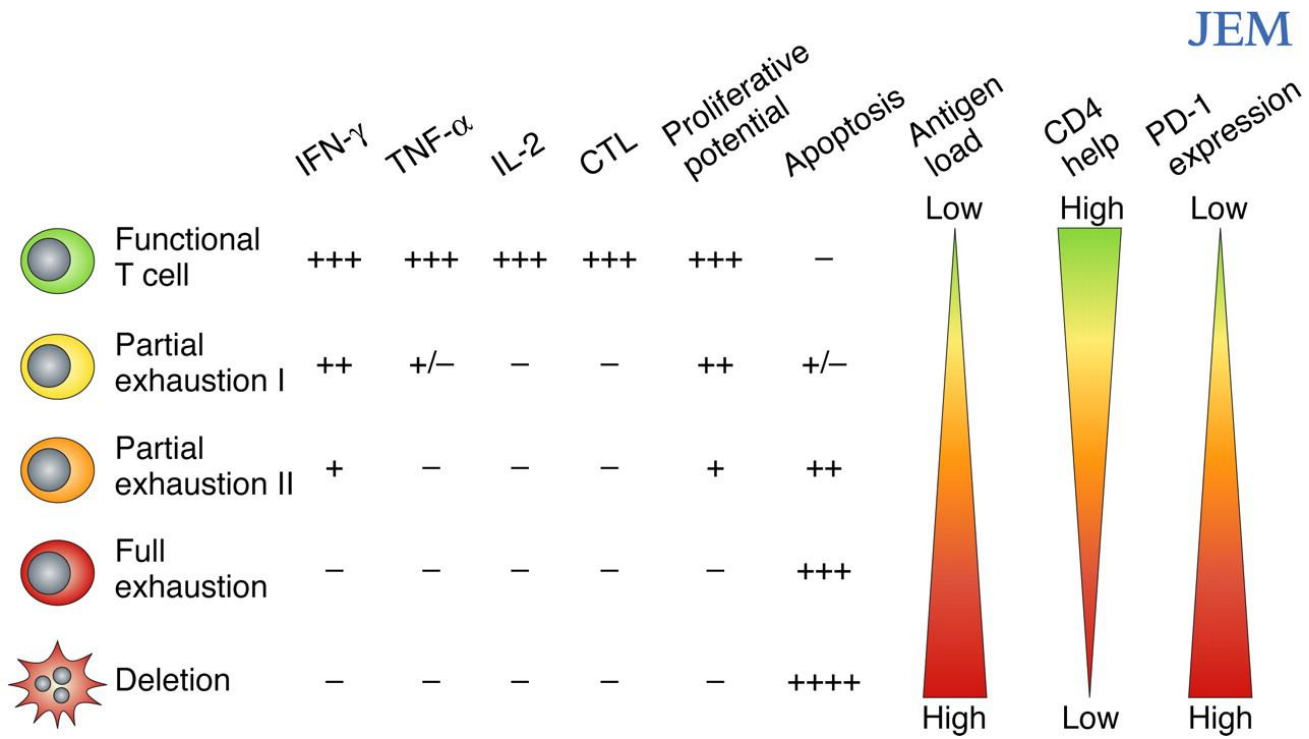


Defining T cell exhaustion as a differentiation state

- T cell differentiation fates like Th1, Th2, effector-memory, etc., all have several common features
  - A distinct functional program (cytokine production)
  - A transcriptional network that drives that program
  - An epigenetic landscape that supports those transcriptional networks
- While still under debate, many in the field believe that exhausted T cells are essentially a distinct subset of T cells rather than a functional state – we will discuss the underlying data for these suppositions

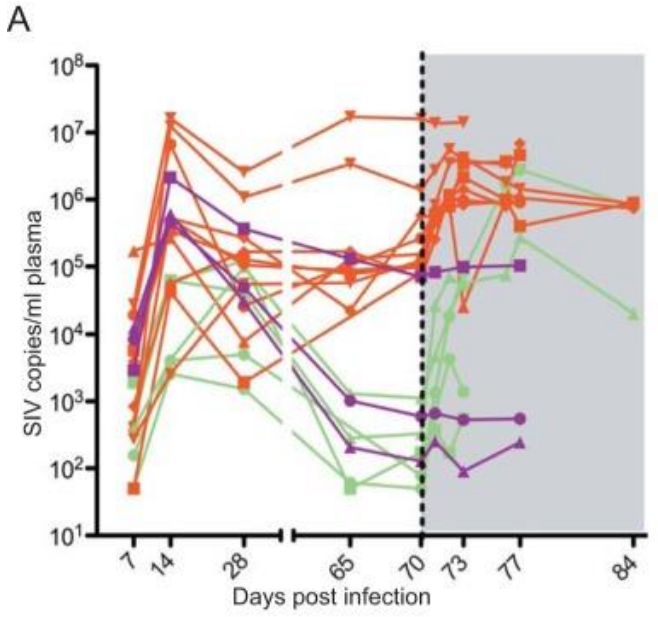
Progression and functional defects of exhausted T cells

- Exhaustion is not strictly hyporesponsive
- Indeed, exhausted T cells still maintain some degree of cytokine production
- But it is the polyfunctionality that is lost: multiple cytokines, killing ability, and proliferative potential that truly defines exhaustion



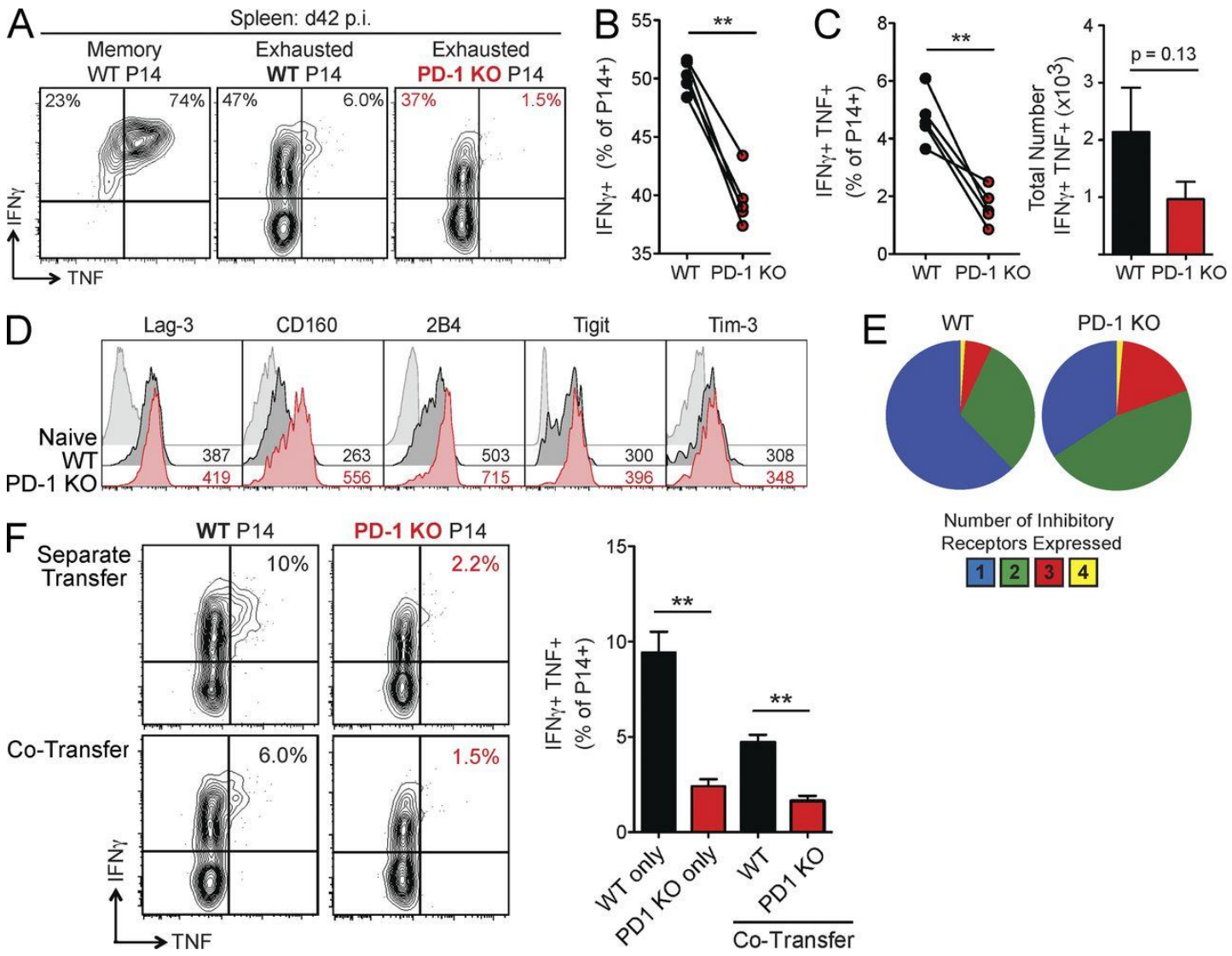
Are exhausted T cells dysfunctional?

- While this seems like an obvious question, it is not a clear answer
- Depletion of exhausted T cells in chronic infections, despite their ‘dysfunctional state’ leads to rampant and lethal viremia
- While similar experiments have not been necessarily done in cancer, these data suggest exhausted T cells retain *some* function
- Indeed, exhausted T cells can still transcribe *Ifng* as well as cytotoxicity genes



So what DRIVES T cell exhaustion?

PD-1 signaling does not *cause* T cell exhaustion



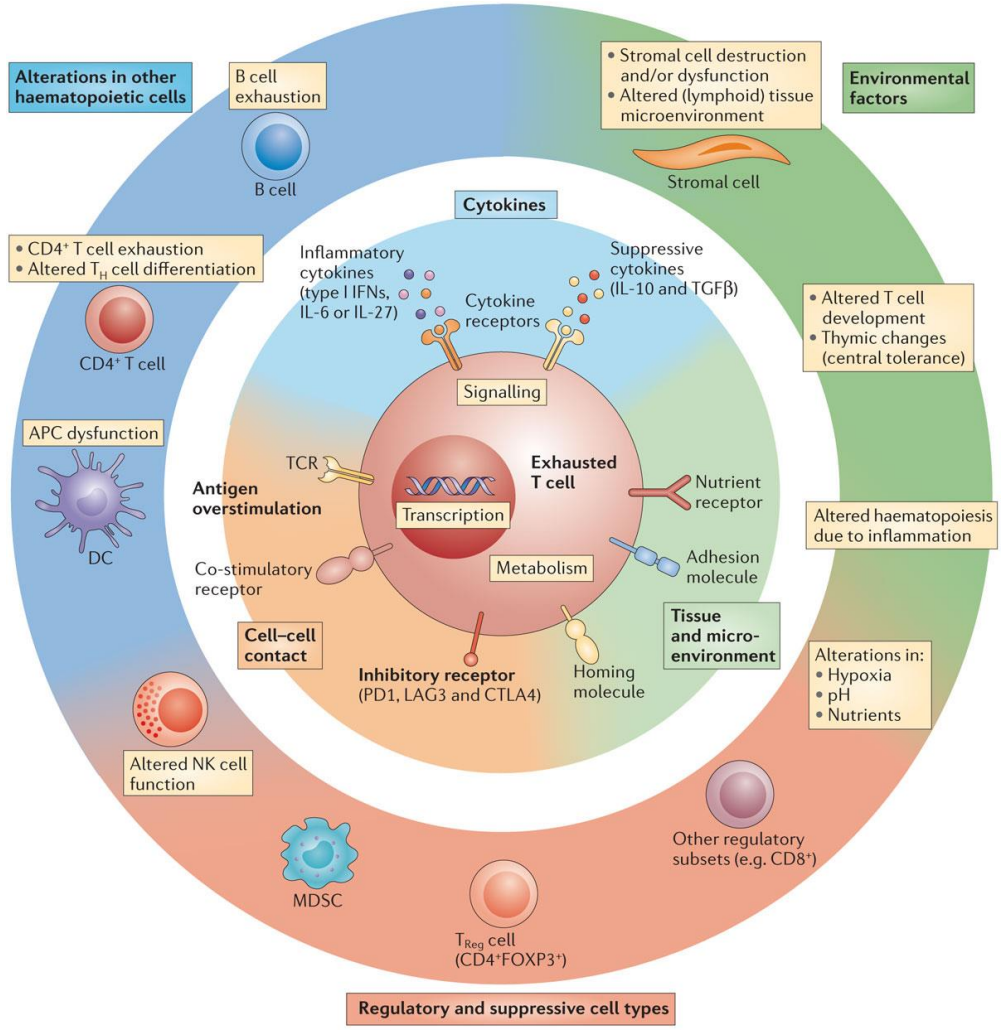
PD-1 deficient T cells still become exhausted in chronic viral infection

In fact, they are even more severely exhausted.

So what does?

Drivers of the exhausted T cell fate

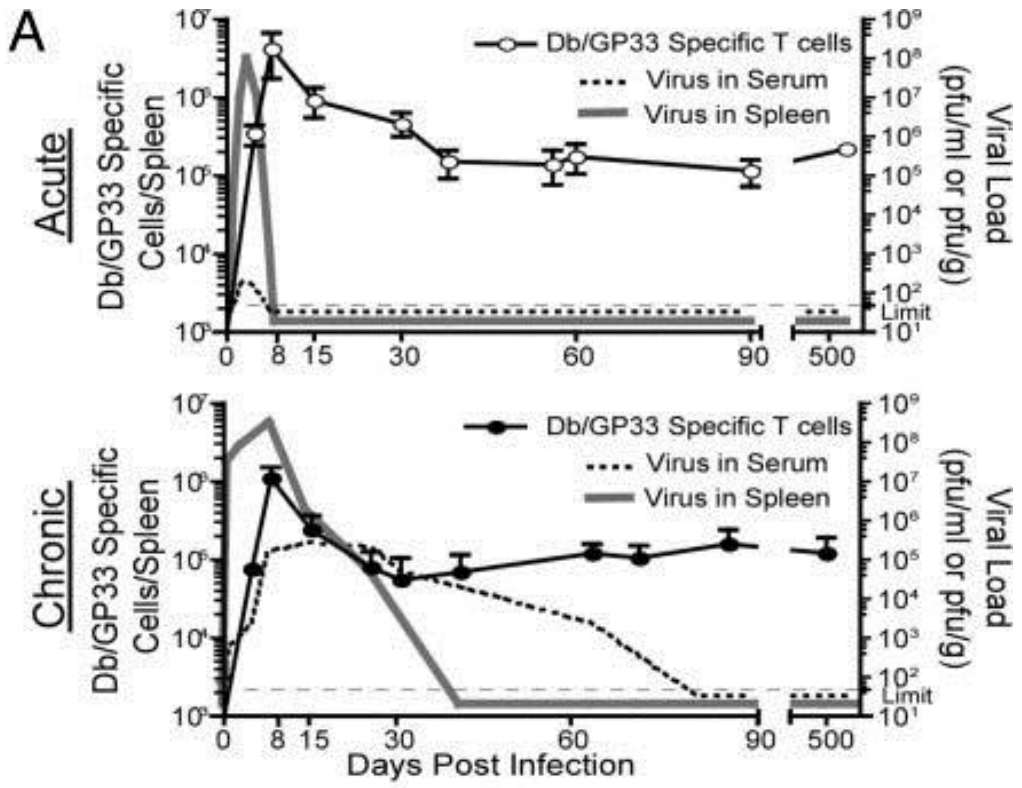
- Antigen – Persistent antigen is associated with T cell exhaustion
- Inflammation – cytokines can play roles in driving certain components of the exhausted cell phenotype
- Microenvironment – Hypoxia, metabolic stress



Nature Reviews | Immunology

Antigen

- The persistence of antigen plays a key role in driving cells to become exhausted
- LCMV is a great system to study this as the differences between the virus strain are so minute
- T cell transfer studies confirmed a role for antigen in this process
  - T cells transferred from acutely infected mice into chronically infected mice become exhausted
  - T cells transferred from chronically infected mice early (1 week post infection) into acutely infected mice become memory
  - T cells transferred from chronically infected mice late (30 days post infection) into acutely infected mice remain exhausted
- TCR transgenic mice specific for model, viral, or tumor antigens have helped find similar types of scenarios (albeit with different kinetics) in tumor systems

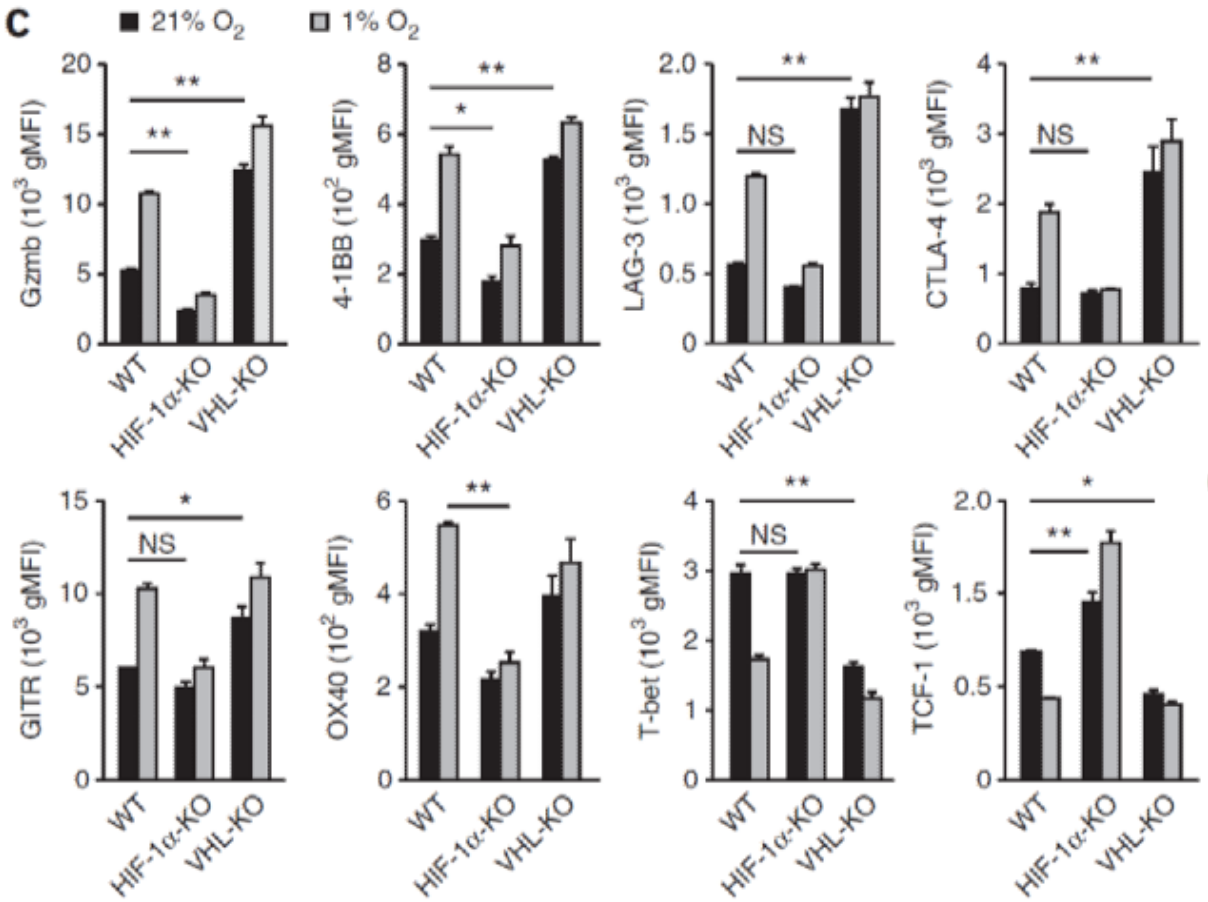


Inflammation

- Use of genetically modified mice in the LCMV system has allowed for direct interrogation of the role of inflammation in exhaustion
- Chronic antigen supposes chronic inflammation, and indeed in chronic infections type I interferon signaling can be overstimulated
- IFNAR-deficient T cells (or anti-IFNAR blockade) result in less chronic infection induced exhaustion
- Tumor necrosis factor (TNF) can also drive exhaustion (TNF blockade results in increased T cell function)
- A host of other factors can drive exhausted like programs, including
  - IL-6, IL-27
  - Treg derived factors like TGFb, IL-10
  - Lack of proper T cell help
  - Exposure to MDSC

Metabolic stress

- Exposure to metabolically stressful conditions has been shown to induce an exhausted-like state
  - Hypoxia: HIF1a drives co-inhibitory molecule expression, but also effector function
  - Low glucose, high lactate
  - ER stress/ ROS production
- How causative these states are in isolation still remains to be determined

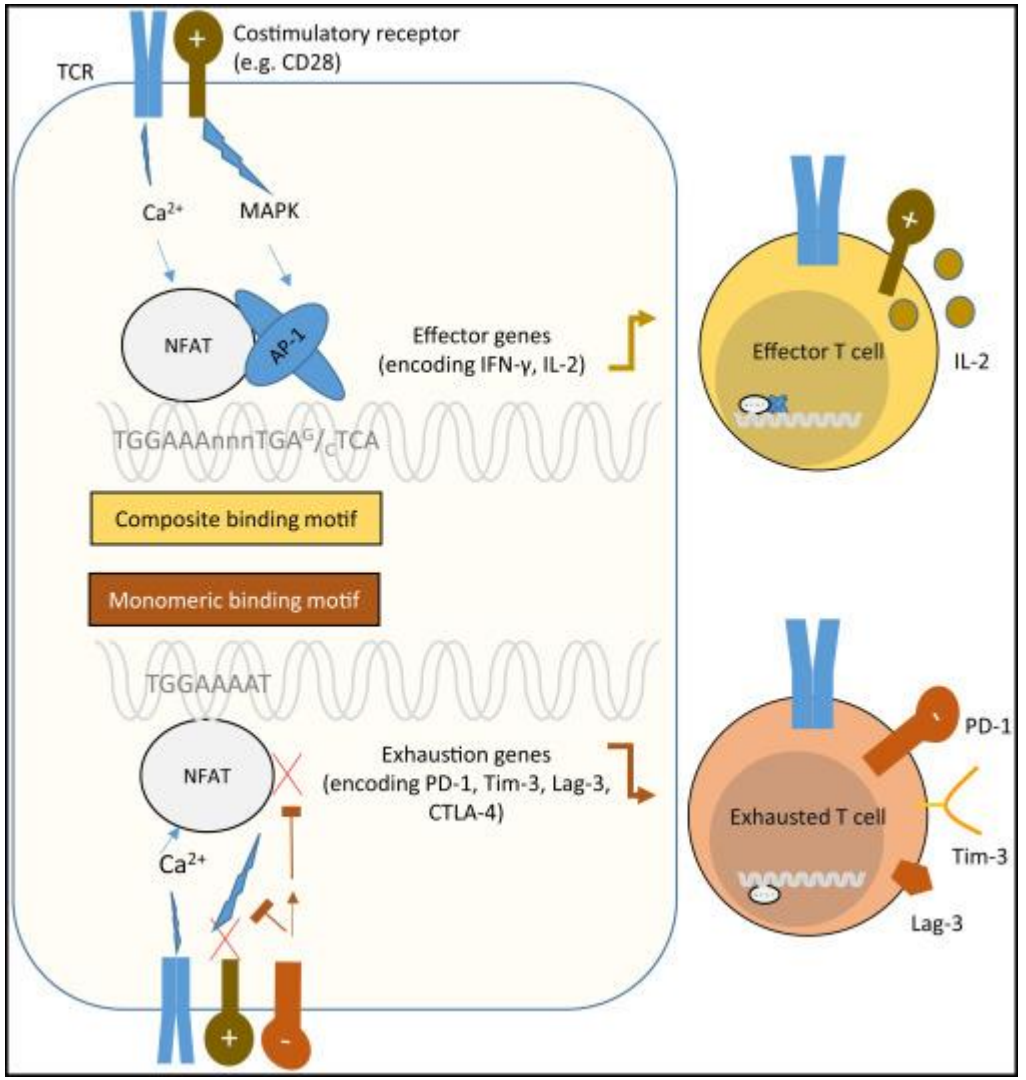


Maintenance of the exhausted T cell fate

- As a differentiation state rather than a functional one, exhausted T cells (especially terminally exhausted ones) are inclined to stay this way
- The biology of T cell exhaustion has been an important scientific endeavor as those targets that arise may be therapeutic targets to modify cellular function for modulation of the immune response

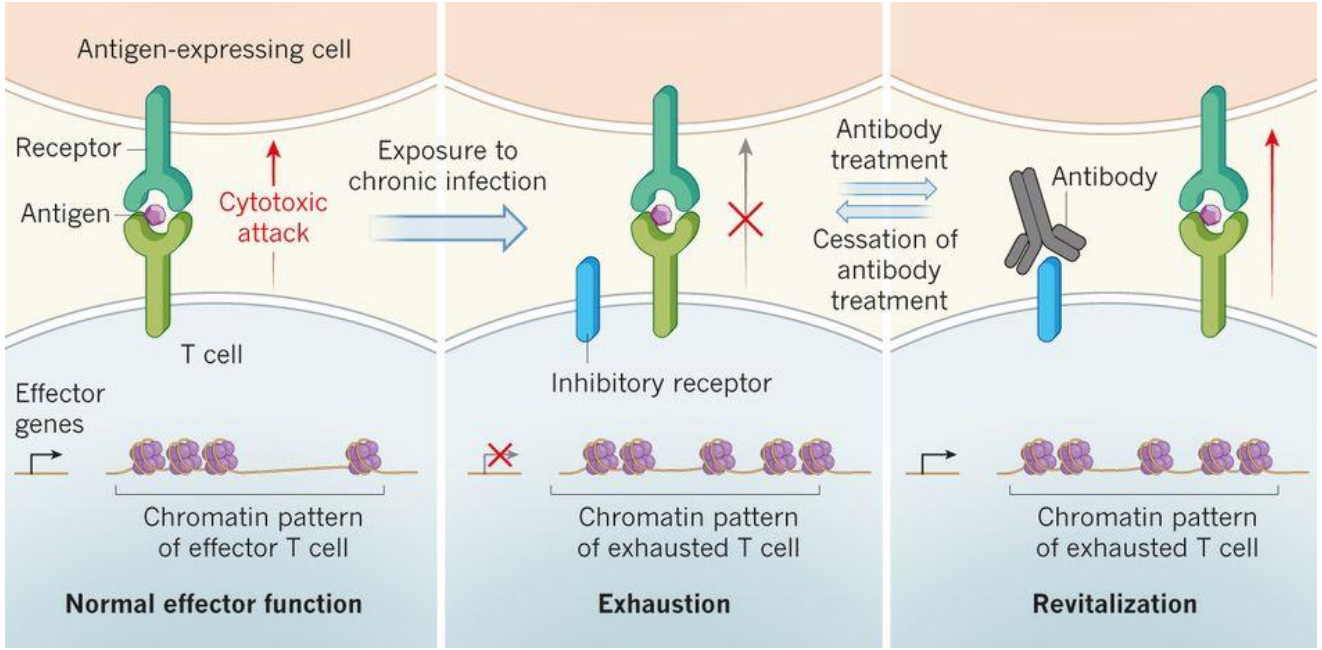
Transcriptional rewiring

- As chronic stimulation is a driver of exhaustion, NFAT, which is downstream of the TCR, is a major culprit in transcriptional changes induced
- NFAT typically partners with AP-1 (induced by costimulation) to program effector gene lineages
- However, chronic stimulation or co-inhibition promotes constitutive NFAT activity which is sufficient for an exhausted like state
- NFAT is not the whole story, however
- Maintenance of the phenotype likely requires transcription factors like
  - Blimp-1
  - T-bet
  - Eomesodermin
  - HIF1a
- However, what has been clear is that the transcriptional program of exhausted T cells does not necessarily explain their dysfunction

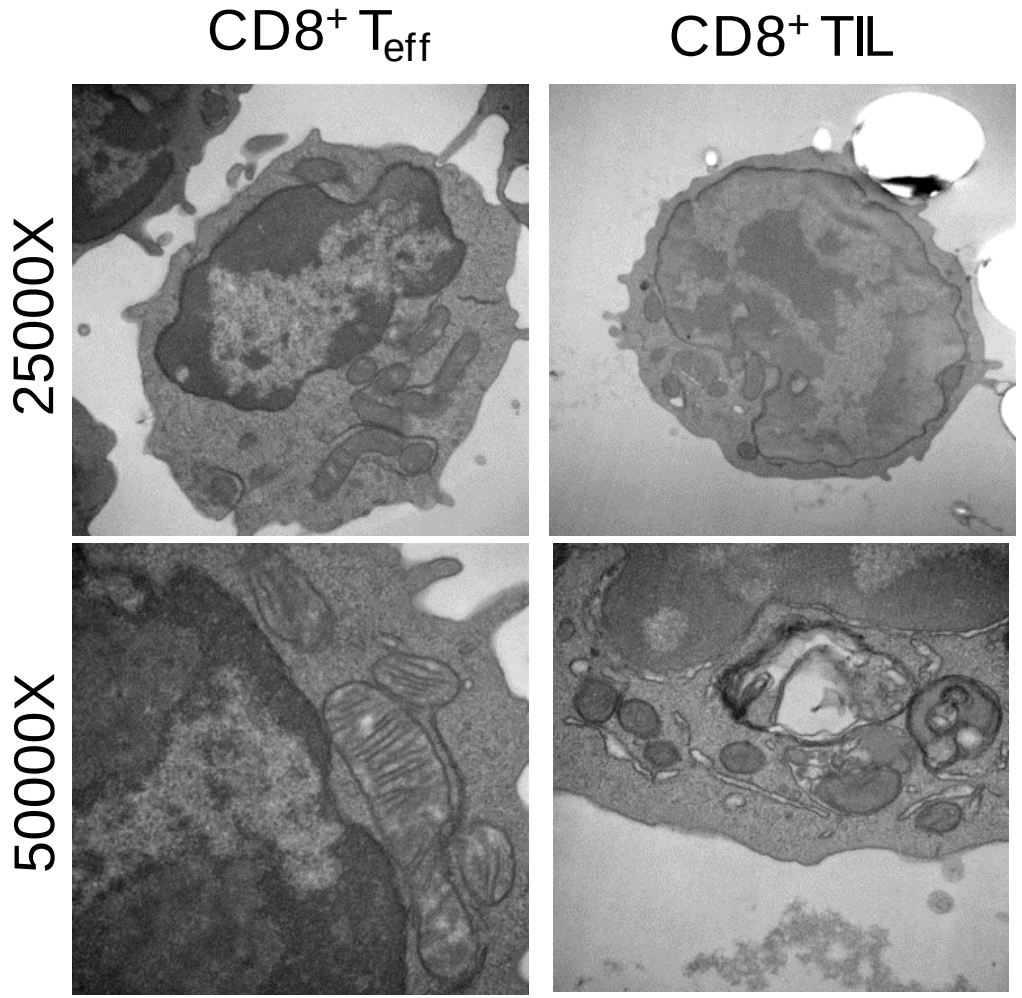


Epigenetics

- Differentiated cells (like Th1 and Th2, CTL and memory, regulatory versus conventional) have epigenetic programs which guide transcriptional machinery
- Several groups using ATAC-sequencing have shown that exhausted T cells have a distinct epigenetic signature
- This signature **is not** changed by PD-1 blockade, which means, inevitably, T cells will ‘slide’ back into their dysfunctional state

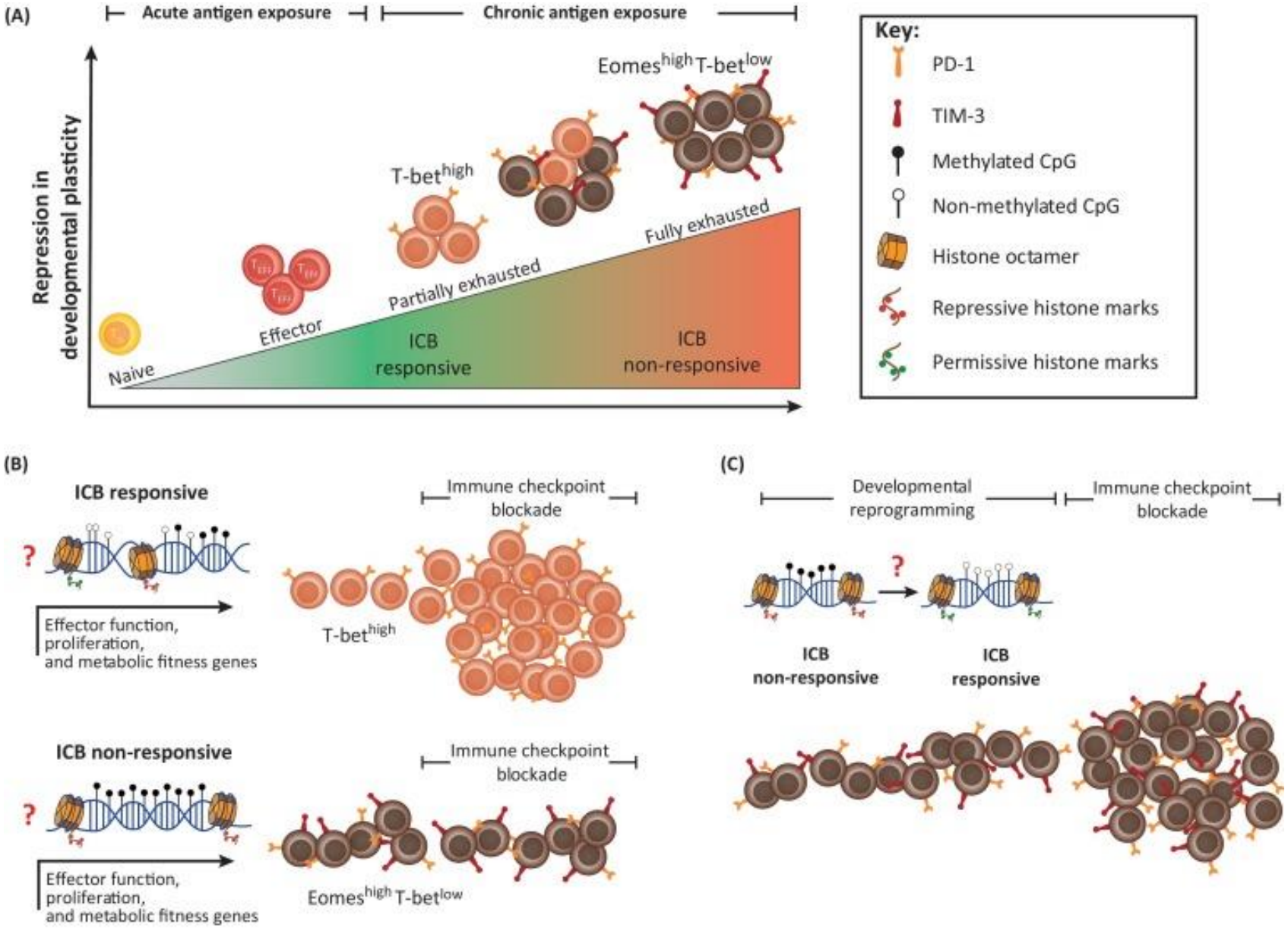


Metabolic insufficiency



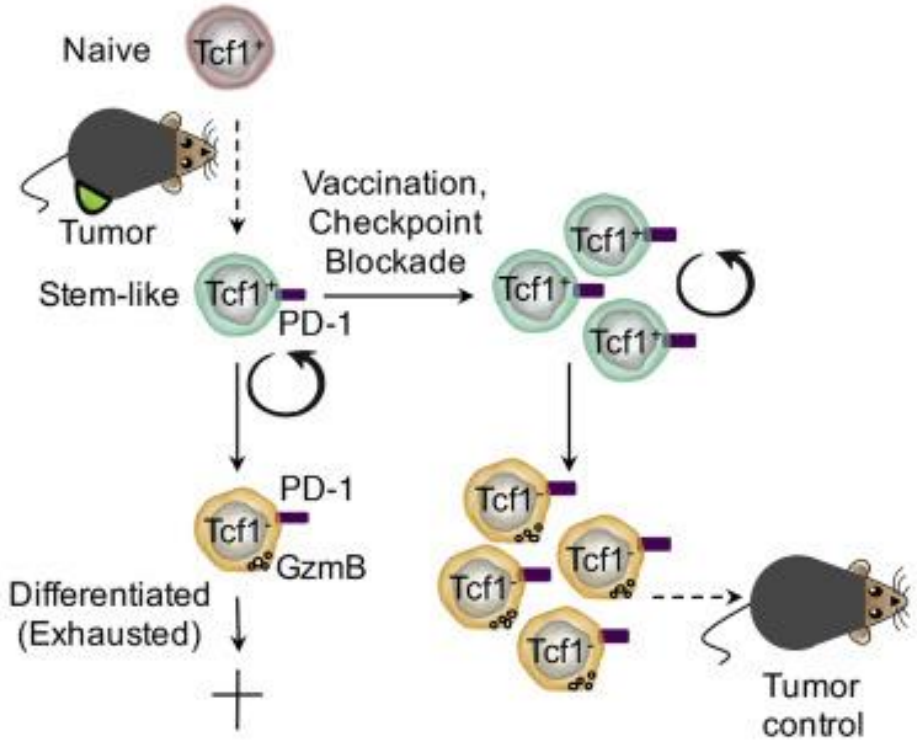
- Exhausted T cells are also characterized by severe metabolic deficiencies
- Exhausted T cells isolated from tumors (and in chronic infection) show repressed glucose uptake and accumulation of dysfunctional mitochondria
- Thus, exhausted T cells are energetically disadvantaged in a nutrient poor environment!

Who does PD-1 blockade affect?



Trends in Molecular Medicine

Who does PD-1 blockade affect?

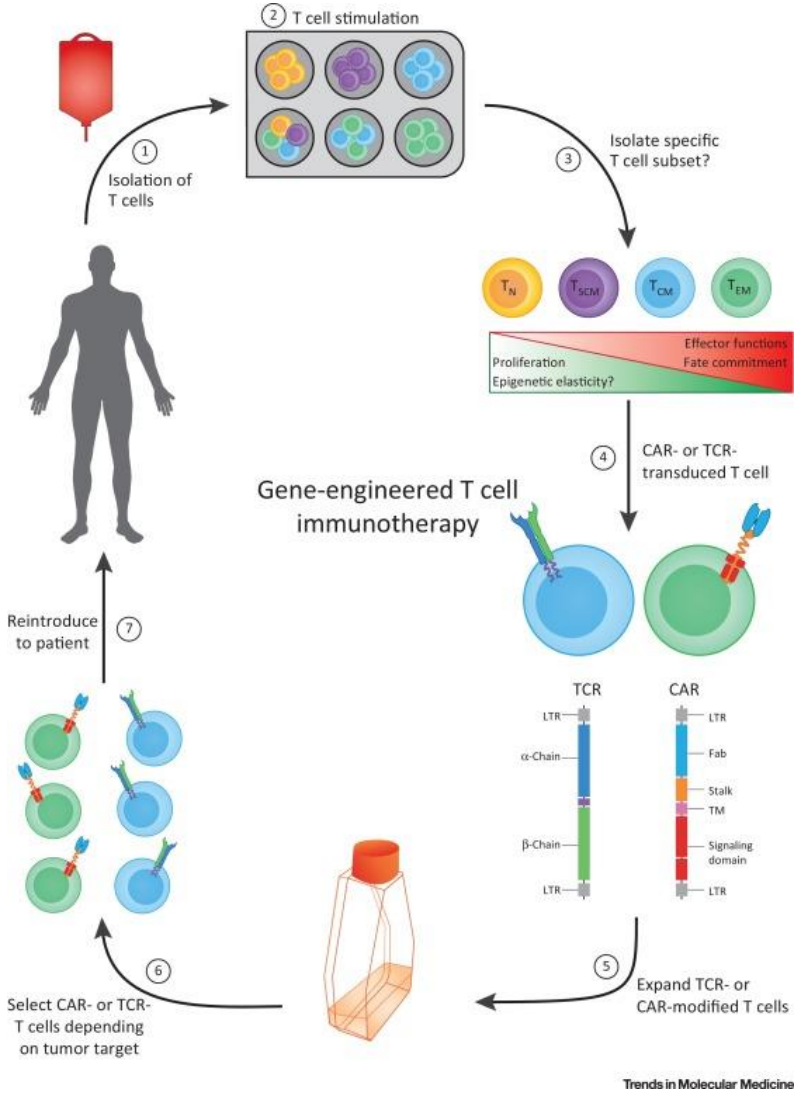


- Single cell sequencing of murine and human tumors indeed confirms this supposition: anti-PD1 likely causes changes to more stem-like PD-1 expressers rather than the terminally exhausted cells
- Thus, PD-1 blockade actually induces changes in cellular fate rather than the immediate function of the cell

What about combinatorial therapies?

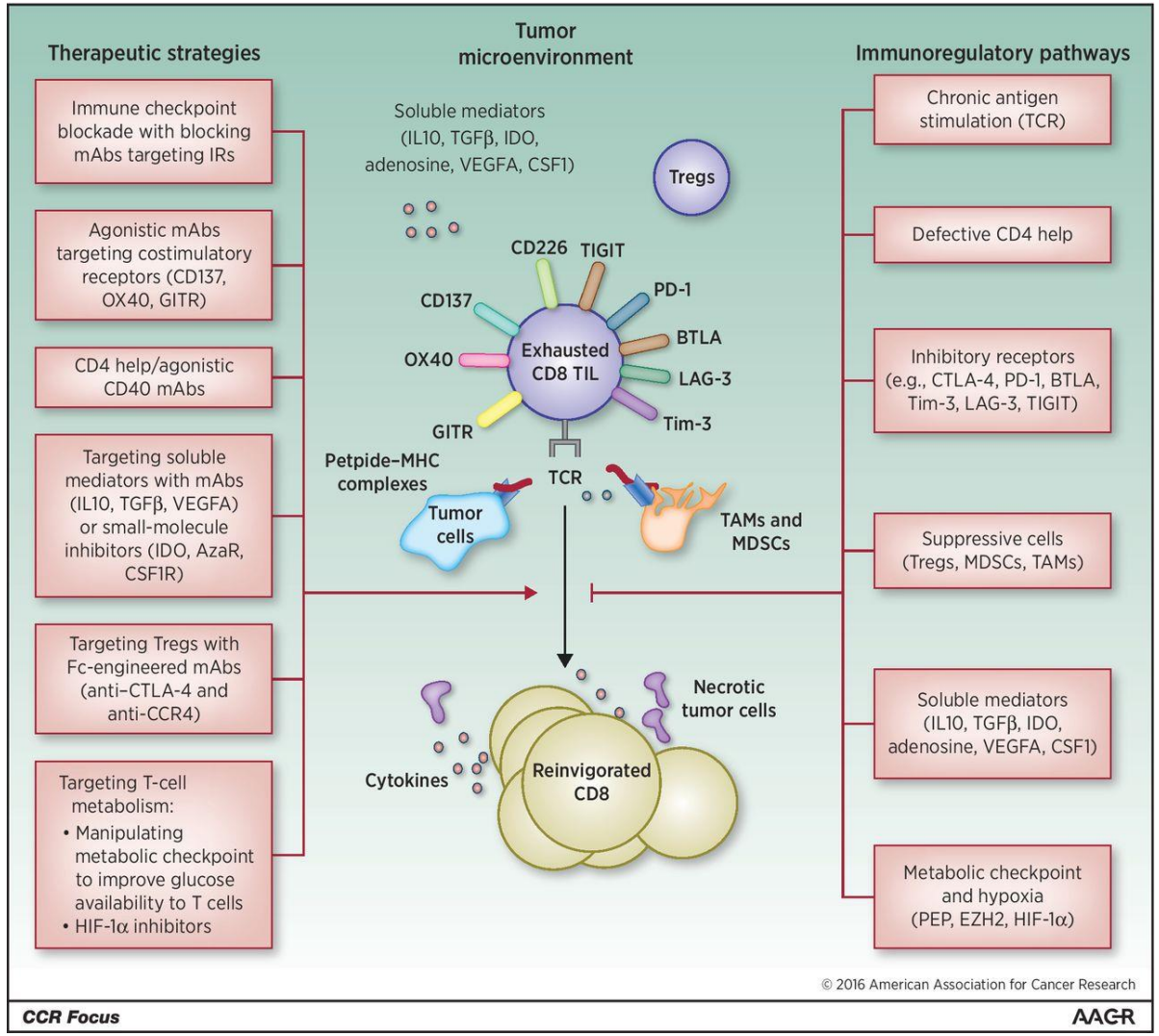
- In ipi/nivo combinations, supposedly, aCTLA-4 may act to prime new T cells, while aPD-1 sustains them/prevents their altered differentiation
- Unfortunately, the newest modalities target molecules whose signaling and downstream biology remain unclear
  - LAG3
  - TIM-3
  - TIGIT
- Understanding the underlying biology to these targets will undoubtedly reveal to us whether these terminally exhausted T cells can be rejuvenated or not

Exhaustion likely affects adoptive cell therapies



- CAR-T cells see a single antigen, and indeed chronic/tonic signaling of these receptors has been shown to lead to metabolic insufficiency and exhaustion
- TIL derive from tumor-reactive, partially or terminally exhausted cells that are cultured in vitro: if their epigenetic identity is maintained even after *ex vivo* culture, it is likely expanded and reinfused TIL will still succumb to the same phenotypes

What is the role of the microenvironment in causing exhaustion?



- Unlike chronic infections, solid tumors have a distinct microenvironment
- Once the tumor microenvironment is established, **the drivers of T cell exhaustion are all concentrated at the tissue site**
- Tumor cells present chronic antigen
- Stromal cells and immature myeloid cells generate persistent inflammation
- Treg cells and MDSC can generate IL-10, TGFb, inhibitory metabolites
- Tortuous angiogenesis combined with high tumor cell metabolism creates hypoxia and nutrient deprivation
- Physical barriers prevent new T cell infiltration

Concluding points for discussion

- T cell exhaustion in cancer arises from a number of tumor- and host-derived factors, including antigen, inflammation, and metabolic stress
- Are tumor-infiltrating T cells exhausted in the same way that those in chronic infections are?
- If exhaustion really is a state of terminal differentiation, are they salvageable?
- Given that these cells are often constitute the majority of tumor infiltrating T cells, what type of measures (artificial, heroic, crazy) could be deployed to reverse these inhibitory states?