

REVIEW

Immunological exhaustion: How to make a disparate concept operational?

Hannah Kaminski , Maël Lemoine , Thomas Pradeu *

ImmunoConcept, CNRS & University of Bordeaux, Bordeaux, France

* thomas.pradeu@u-bordeaux.fr

Abstract

In this essay, we show that 3 distinct approaches to immunological exhaustion coexist and that they only partially overlap, generating potential misunderstandings. Exploring cases ranging from viral infections to cancer, we propose that it is crucial, for experimental and therapeutic purposes, to clarify these approaches and their interconnections so as to make the concept of exhaustion genuinely operational.



Author summary

In this essay, we have written a critical review on immunological exhaustion. We believe that this widely used concept often remains in fact imprecise because there exist 3 different approaches to exhaustion, namely, in terms of dysfunction, cause, and marker, and those are not sufficiently well distinguished and articulated in most scientific papers. We also propose to talk about “exhaustion” for and only for the phenomena in which all 3 approaches are aligned. This can be called the “convergence strategy”: T cells would be described as “exhausted” if and only if they are simultaneously dysfunctional, express given markers such as programmed cell death protein 1 (PD-1), and induced by a specific cause such as chronicity. This strategy could be perfectly reasonable, and we see much value in adopting it, because it would force everyone to be more specific and rigorous when talking about exhaustion. Clarifying the characteristics of cells defined as “exhausted,” and using a convergent strategy to define T-cell exhaustion could have major experimental and clinical consequences, including for viral infections such as the Coronavirus Disease 2019 (COVID-19).

Three approaches to exhaustion

Despite previous mentions to “exhaustion” in the immunological literature [1,2], the founding period for modern uses of the notion was the 1990s, during which exhaustion was defined both causally and functionally, focusing on specific CD8+ T cells during murine chronic lymphocytic choriomeningitis virus (LCMV) infection. “Exhausted” T cells are cells that, when exposed chronically to high quantities of antigen, are activated and proliferate, before becoming dysfunctional, i.e., unable to eliminate the virus. For Zinkernagel and colleagues [3], exhaustion is defined by the non-elimination of the virus, due to the peripheral deletion of all

OPEN ACCESS

Citation: Kaminski H, Lemoine M, Pradeu T (2021) Immunological exhaustion: How to make a disparate concept operational? *PLoS Pathog* 17(9): e1009892. <https://doi.org/10.1371/journal.ppat.1009892>

Editor: Salomé LeibundGut-Landmann, Universität Zurich, SWITZERLAND

Published: September 23, 2021

Copyright: © 2021 Kaminski et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme - grant agreement n° 637647 – IDEM (TP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

the virus-specific cytotoxic T cells, a concept validated a few years later [4]. For Ahmed and colleagues [5], exhausted cells are dysfunctional because their capacity to trigger an effector response against the virus is reduced, but these cells are maintained in the body. Overall, exhaustion has classically been defined both by dysfunction (these T cells fail to do what effector T cells are expected to do) and by a double cause (high viral load and chronicity). Following the work of Zinkernagel and Ahmed, the concept of exhaustion has most of the time been applied to CD8 T cells, although it is also sometimes attributed to other immune cells (especially CD4 T cells) (e.g., [6]). Both Zinkernagel's and Ahmed's groups worked on chronic LCMV infection in mice, which rapidly became, and still is today, the standard model for understanding exhaustion.

In subsequent research until present day, 3 approaches to immunological exhaustion have coexisted, with often unclear connections (Fig 1). The first approach primarily defines as exhausted the cells that present the same cellular dysfunction (typically, the absence of an expected effector response). The second approach primarily defines as exhausted the cells that are produced by a given cause (typically, but not necessarily, chronic exposure to an antigen). Finally, the third approach primarily defines as exhausted the cells that present the same molecular markers (typically, programmed cell death protein 1 [PD-1]). One difficulty is that authors tend to overlook specifying which approach they have in mind when they qualify cells as "exhausted." A second even more serious difficulty is that authors often act as if these 3 approaches necessarily aligned (i.e., as if the 3 properties always occurred jointly), when in fact they don't. As we will see, even the seemingly consensual view that antigen chronicity is the leading cause of exhaustion is questionable, including in the historically paradigmatic LCMV example. More recently, a subset of stem-like CD8+ T cells has been identified among exhausted T cells and presented as a much more rigorous characterization of exhaustion, but, as we will see, the nonoverlap problem also applies to this subset [7–10].

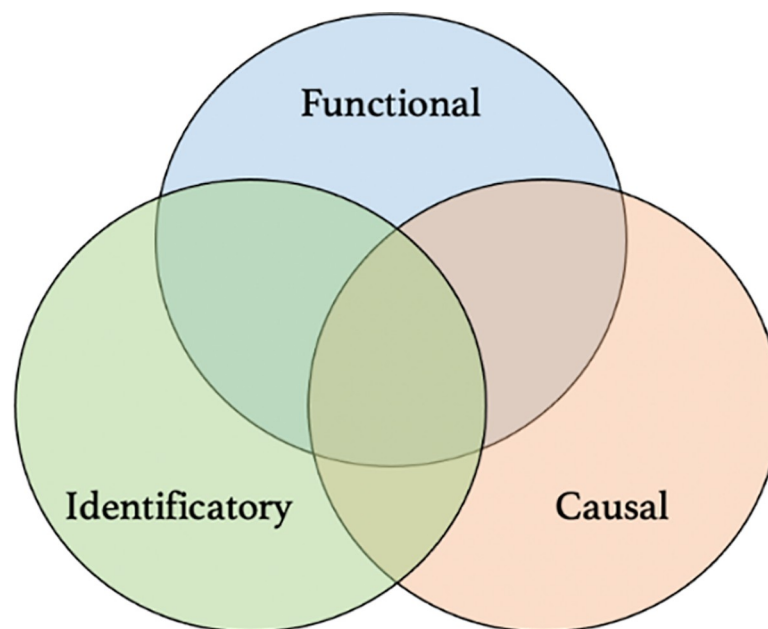


Fig 1. Three different approaches to immunological exhaustion. These approaches use different criteria and do not always overlap.

<https://doi.org/10.1371/journal.ppat.1009892.g001>

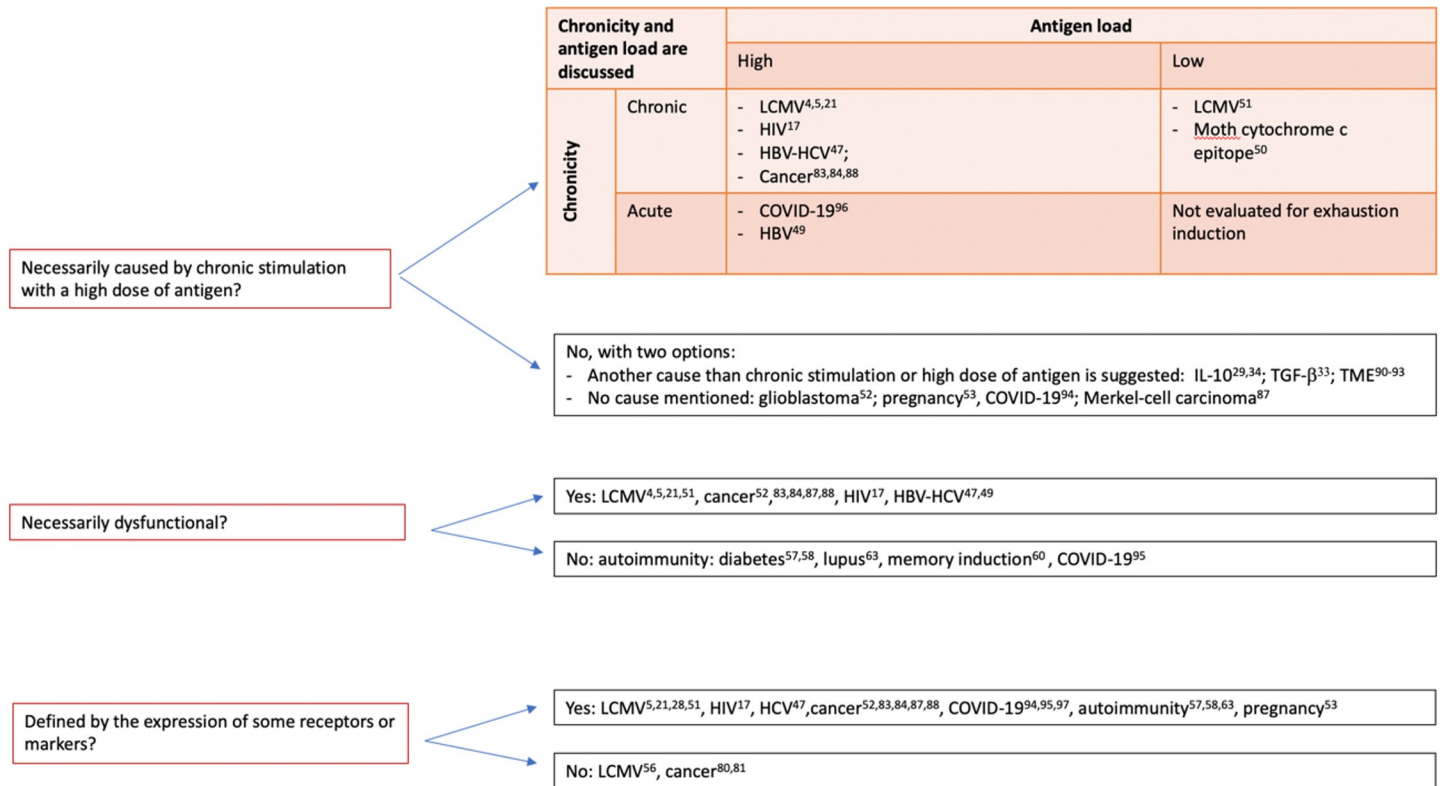


Fig 2. Key controversies about immunological “exhaustion”. COVID-19, Coronavirus Disease 2019; HBV, hepatitis B virus; HCV, hepatitis C virus; IL, interleukin; LCMV, lymphocytic choriomeningitis virus; TGF-β, transforming growth factor beta; Tumor Micro Environment (TME), xxx.

<https://doi.org/10.1371/journal.ppat.1009892.g002>

Below, we give several examples of nonoverlap, and we insist on the importance of both distinguishing and combining these 3 approaches to put together a precise and operational account of exhaustion.

In our view, the lack of articulation between the 3 approaches distinguished above is the prime explanation for the current ambiguities and disagreements around the notion of exhaustion, as exemplified recently in the various and often competing positions expressed by 19 experts [11]. Fig 2 sums up the main controversies about exhaustion.

Because we propose that it is essential for immunologists to distinguish the 3 approaches and make explicit in each experimental or therapeutic context which approach(es) they have in mind, we will try to present as precisely as possible these 3 approaches.

The functional approach: Exhaustion is a cellular dysfunction

Originally, exhausted T cells were understood as dysfunctional. What is meant by “dysfunction” here is that some T cells, after activation and proliferation, do not fulfill the functions they are expected to perform as effector T cells—typically, they fail to eliminate infected cells and control the virus. As originally described, antigen-specific T cells become “dysfunctional” during the chronic phase of high viral load infections, with progressive loss of interleukin (IL)-2, then tumor necrosis factor alpha (TNFα), and, finally, interferon gamma (IFNγ). Authors have called these cells “dysfunctional” because they compare their functions in the context of a high-dose persistent chronic viral infection with the functions of the same cells in the context of acute viral infection when the virus is cleared [1].

In the original sense of “exhaustion” and especially for Zinkernagel’s team, the dysfunction that is characteristic of exhaustion can be defined independently of any cause or marker. Exhaustion is understood as the non-elimination of the virus due to the peripheral deletion of virus-specific cells. This constitutes a genuine functional characterization of exhaustion. Within this approach, saying that the cause of exhaustion is the exposure to high doses of a chronic antigen is therefore not circular: This cause is offered as the best possible explanation for an independently defined phenomenon.

The dysfunction associated with exhaustion has often been conceived in contrast to another dysfunctional state, namely “anergy.” The consensus is that anergy results from an absence of signal 2, and, therefore, a failure to become activated, while exhaustion occurs after T cells have been fully activated and have proliferated [12–14].

It is important to characterize as precisely as possible the nature of the dysfunction thought to be characteristic of exhaustion. For example, although this dysfunction has sometimes been conceived as involving an incapacity for maintenance and expansion [15–17], some T-cell populations (the stem-like CD8⁺ exhausted T-cell subset [18]) can persist and expand in chronic infections [13]. We now know that the exhaustion of such stem-like cells is, at least in some situations, reversible [12], contrasting with the long-held view that exhaustion would necessarily be irreversible [3,19–21]. The reversibility seems to concern especially T cell factor 1 (TCF1)⁺ cells. These PD-1⁺CD8⁺ T cells resemble stem cells during chronic LCMV infection, undergoing self-renewal and also differentiating into the terminally exhausted CD8⁺ T cells [9]. However, interferon regulatory factor 4 (IRF4) inhibition also seems able to induce TCF1 and leads to exhaustion reversibility [22].

“Exhausted” cells transferred into a naive host can reexpand and protect it against the same pathogenic challenge [23], suggesting that such cells are not irreversibly “dysfunctional,” and this restoration is now known to occur through the stem-like exhausted cells [9]. However, the dysfunction of stem-like cells concerns maintenance, expansion, and differentiation but not cytokine production (IFN γ) and cytotoxicity (granzyme B) since the terminally exhausted cells originating from the stem-like ones have the highest cytotoxic potential [9].

Is exhaustion always dysfunctional? There are important disagreements on this issue, some authors considering that exhaustion is by definition dysfunctional, while for others, exhaustion could be functional in some contexts [11,23,24]. One major limitation is that, in general, exhausted T cells are said to be “dysfunctional” with a unique and preexistent idea of “function” in mind—namely, the function to eliminate the virus. This expected and unfulfilled function remains in almost all cases the unique focus of the observer, which means that other active functions, such as immunoregulation or tissue repair, for example, are generally not explored. This makes it impossible to determine if, by becoming “exhausted,” cells lose some functions while simultaneously acquiring other functions. Major examples of contexts where so-called “exhausted” T cells could in fact play essential functional roles include the limitation of immunopathology in infection [25] and the regulation of autoimmunity [26].

The causal approach: Exhaustion as resulting from specific causes

A second major approach is to say that exhausted cells are cells that are produced by a particular cause, typically antigenic chronicity and/or high antigen load. In the original LCMV model [3,5], both chronicity and antigen load were considered essential causes leading to exhaustion.

In subsequent work, antigenic chronicity remained an often mentioned typical cause leading to exhaustion [21,27]. Naturally, an important challenge when saying that exhaustion results from chronic exposure to an antigen is to determine what is meant by “chronic,” i.e., what the exact time course of exhaustion appearance is. Despite the general consensus that

exhaustion is related to antigen chronicity, recent results have shown that this connection does not always hold. Although exhaustion has been classically observed during the chronic phase of uncleared viruses, it has been shown recently that, as early as 9 days postinfection of C13 LCMV, CD8 T cells lose their ability to make TNF α and IL-2 [28,29], which seems a very short time to refer to some type of “chronicity.” It has been suggested that thymocyte selection associated high mobility group box (Tox) determines early the T-cell exhaustion fate, but this has not been formally demonstrated yet [28]. A high dose of C-13 (10^6), as opposed to a low dose of the same virus (10^2), leads to exhausted cells by day 8 [9], suggesting that the antigenic load prevails over the chronicity of antigen exposure in T-cell exhaustion.

A second cause said to trigger exhaustion is indeed high antigen load. As previously said about the historical LCMV model, the claim that T cells were exhausted in C-13 by contrast to the Armstrong strain was based on the fact that the viral load was both higher and chronic [5,21]. The insistence on the role of a high antigen load also has roots in the concept of “immune paralysis.” This concept, widely used in the 1960s to 1970s [2,30,31], proposed that the immune system could become inoperant when confronted with high doses of antigen [21,28]. Later, some papers insisted on the crucial importance of high antigen load in the characterization of exhaustion, with or without an accompanying mention of chronicity [21,29,32]. A key challenge for this approach is to determine what quantity of antigen constitutes a “high dose” [21].

Importantly, other causes of exhaustion, beyond chronicity and antigen load, have been suggested, including contextual causes (Fig 2). One such additional cause is the cytokine environment, with a typical focus on IL-10 and TGF- β (which tend to favor exhaustion) [29,33,34] as well as IL-21 (which, in contrast, tends to inhibit exhaustion) [35]. Incidentally, the impact of the cytokine environment on the functionally defined exhausted phenotype of CD8 T cells demonstrates that these cells are not intrinsically “exhausted”; instead, exhaustion in the functional sense appears to be context dependent. Exhaustion is also influenced by causes at the cellular level, including both regulatory T cells [36,37] and myeloid-derived suppressor cells [38].

The identificatory approach: Exhaustion as the cellular state associated with the expression of particular molecular markers

According to the third approach, exhaustion is the cell state associated with the expression of particular molecular markers—classically inhibitory receptors. Because there are many of them, only the most discussed in the scientific literature are mentioned here.

PD-1 has been the most extensively studied marker of exhaustion. It is considered as characterizing exhausted T cells in both chronic infection and cancer and associated with dysfunction in both cases [39,40]. In addition to PD-1, there are other receptors often considered as markers of exhaustion, often but not always in association with PD-1 (lymphocyte activation gene 3 [LAG3], T cell immunoglobulin and mucin domain-containing protein 3 [TIM3], T cell immunoreceptor with Ig and ITIM domains [TIGIT], cytotoxic T-lymphocyte antigen 4 [CTLA-4], etc.).

Some transcription factors have been interpreted as favoring exhaustion, for example, B lymphocyte-induced maturation protein 1 (Blimp-1), Eomesodermin (Eomes), Tox, and IRF4 (reviewed in [41]). During chronic infection, Eomes has been correlated with the “more terminal T_{ex} subset” [42]. Tox has been considered as a key transcription factor of the T-cell exhaustion program, since it was highly expressed in T cells during C-13 LCMV infection, whereas it was only transiently expressed at low levels during acute infection with Armstrong [28]. Moreover, T-cell exhaustion in cancer and chronic infection mainly relies on the presence of Tox,

itself driven by chronic TCR stimulation and nuclear factor of activated T cells (NFAT) activation and associated with the expression of other transcription factors that are required for exhausted T cells (TCF1 and Eomes), as well as with inhibitory receptors and decreased function [28]. IRF4 contributes to inducing inhibitory receptors and leads to decreased functionality of CD8+ T cells during the chronic phase of LCMV infection and during cancer [22,43,44].

Finally, exhausted cells display metabolic changes such as inhibition of aerobic glycolysis due to glucose transport limitation and consumption by cancer cells, mitochondrial dysfunction, and oxygen deprivation [45], which, in turn, decreases cytokine production [46].

The incomplete overlap between the 3 approaches to exhaustion

A common attitude is to assume that the 3 approaches to exhaustion generally align: Dysfunction would be produced by well-identified causes such as chronicity and/or high antigen load, and it would be associated with the expression of well-identified markers such as PD-1. In reality, though, these 3 approaches often fail to overlap. A lot of data suggests that exhaustion in the functional sense does not always causally result from antigen chronicity. Exhausted T cells have been described in chronic infection with LCMV, HIV [17], hepatitis B virus (HBV), and hepatitis C virus (HCV) [47], but not in cytomegalovirus (CMV), for example. Even if the 2 different kinetics of viral load have been mentioned as an explanation for the 2 distinct situations (exhaustion in the case of LCMV and inflation in the case of CMV), a better understanding of the cause involved is needed (CMV is thought to give series of low and short multiple replication periods contrasting with high chronic level of replication during LCMV, but studies on CMV suggest that the virus could still replicate actively in the tissues despite its absence in peripheral blood [48]). Moreover, the analysis of the profile evolution of CD8+ T cells in several infections shows that exhaustion in the sense of a dysfunctional phenotype occurs during the acute phase of C-13 LCMV infection, but not in other infections such as influenza, vesicular stomatitis virus (VSV) or *Listeria monocytogenes* [28], and HBV [49]. In a number of cases, antigen chronicity has been shown to be only partially related to exhaustion in a functional sense: Depending on the epitope, antigen load [27,50], and duration of infection, antigen-specific CD8 T cells responding to chronic antigen exposure may be fully functional, partially exhausted, fully exhausted, or physically deleted [21]. In other instances of exhaustion, we simply don't know if antigenic chronicity plays a role or not. For example, in patients with non-small cell lung cancer, hepatocellular carcinoma, and glioblastoma [28,51], or during pregnancy [52], there is no kinetic approach that would establish whether the chronic exposure to tumor or fetal antigens played a role in the induction of T-infiltrating or peripheral blood lymphocyte exhaustion.

Another significant (and related) challenge is that exhaustion in the functional sense is not always caused by exposure to high doses of antigen. It has been recently shown that, in different infections, the adoption of an "exhausted" dysfunctional phenotype by antigen-specific CD8+ T cells occurred before the viral outcomes diverged, suggesting that viral load was not a primary driver of differential expression [28].

Regarding the markers used to define exhaustion, lack of overlap can also be observed. First, although PD-1 is the most often mentioned inhibitory receptor associated with exhausted T cells, it is certainly not a specific marker of exhaustion, as it is expressed after acute TCR activation [53]. The expression of PD-1 by non-exhausted cells is a widely recognized phenomenon [27]. Minimally, this forces us to recognize that PD-1 might be a necessary, although insufficient, marker of exhaustion and that exhaustion corresponds to a cluster of several markers rather than just 1 marker [54]. Saying that exhaustion is associated with a cluster of markers without being able to specify which is unsatisfactory. Moreover, PD-1 is not

always required for the induction of exhaustion, and some features of exhaustion can even be more severe when PD-1 is absent [15]. Finally, the role of PD-1 has been highlighted in many other contexts than exhaustion, including autoimmunity [55], central and peripheral tolerance [56,57], acute infection for memory response [58], and balance between efficient anti-infectious defense and immunopathology [59]. In those contexts, PD-1 was not always associated with cellular dysfunction, and it was even sometimes associated with an increased memory response [57,58]. Thus, the function of PD-1 is interpreted differently depending on where and when it has been studied—sometimes as a sign of “exhaustion,” sometimes not. It seems that the most appropriate description would be that PD-1 is expressed in contexts of inhibition, rather than contexts of exhaustion per se, in so far as “exhaustion” is generally used when PD-1 is expressed in a context that is detrimental to the organism, but not when the context is beneficial (e.g., in peripheral tolerance during autoimmunity [60] or during T-cell memory formation [58]). Conversely, anti-PD1 therapy has been associated with the development of autoimmune diseases such as type 1-like diabetes [61].

Transcription factors also have ambivalent roles depending on the context. For example, Eomes has been associated with exhaustion during the chronic phase of infection, but it is up-regulated during acute infection, favoring effector molecule production (IFN γ), IL-15R β , and memory development [42,62]. Tox expression, highlighted as a key component of the exhaustion program, can in fact be already observed at day 4 of infection before antigen loads differ [28], which suggests that neither the chronicity nor the viral load are primary drivers of its expression. Moreover, the deletion of Tox restores the polyfunctional profile transiently but not in the long term, weakening the idea that this marker necessarily overlaps with dysfunction. Since the cause mentioned by the authors was chronic TCR stimulation, they hypothesized that the TOX program prevents overstimulation of T cells and immunopathology [39,63], which constitutes an alternative function rather than a dysfunction properly speaking. Consequently, the role of the differential expression of Tox in C13, Armstrong, and other microbial infections remains poorly understood.

IRF4 has been described both in LCMV infection and in allotransplantation, with a potential dual role. It shows a pro-exhaustion role during LCMV and cancer. In contrast, the deletion of IRF4 in CD4 T cells resulted in dysfunction and graft tolerance, which means that, when expressed, IRF4 represses PD-1 and Helios [64] and favors reactivity to the allograft.

Finally, the metabolic changes observed in exhausted T cells can also occur in the tumor microenvironment through causes distinct from antigen chronicity and high antigen load [65].

The upshot is that, despite the frequent tendency to consider the 3 approaches to immunological exhaustion to be aligned, it is often not the case. Some situations instantiate “exhaustion” from the functional viewpoint but are not associated with the classic molecular markers of exhaustion and/or the classic causes of exhaustion. This absence of convergence between the 3 approaches can be extremely problematic, as we can illustrate it now with the examples of cancer and Coronavirus Disease 2019 (COVID-19).

Two illustrations of issues raised by incomplete overlap: Cancer and COVID-19

The fact that the 3 approaches to exhaustion do not always conceptually overlap can generate significant issues at the experimental and clinical levels.

The application of the notion of “exhausted T cells” to cancer is particularly telling. The aim of cancer therapies based on immune checkpoint inhibitors is to target dysfunctional T cells in cancer by reversing their state of tolerance to the tumor [66,67]. In recent years, many have

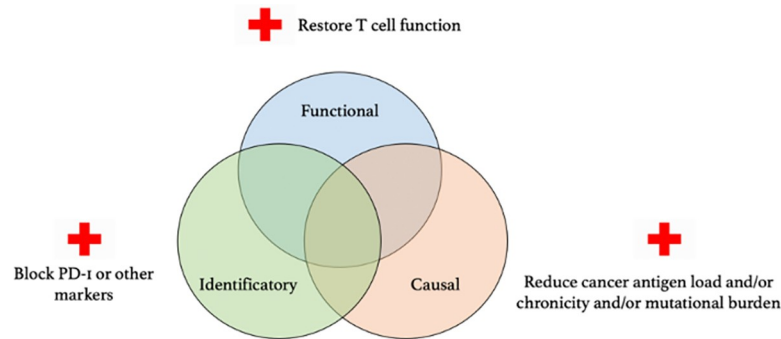


Fig 3. Targeting “exhaustion” in cancer therapies means different things depending on the approach to immunological exhaustion one adopts. PD-1, programmed cell death protein 1.

<https://doi.org/10.1371/journal.ppat.1009892.g003>

connected the literature on immunotherapies with the preexisting literature on immunological exhaustion and claimed that targeting dysfunctional T cells in cancer amounted to targeting exhausted T cells [41, 68–70]. Importantly, most studies on T-cell exhaustion in cancer have been done in mice (e.g., [67,71]), but some have been done in humans (e.g., [72–74]).

The claim that targeting dysfunctional T cells in cancer is equivalent to targeting exhausted T cells is, however, problematic for at least 2 fundamental reasons. First, for the clinician, targeting “exhausted T cells” will have a very different meaning depending on whether what is targeted is the dysfunction itself, the underlying causes, or the markers of exhaustion (Fig 3). Acting as if the 3 approaches always overlapped in cancer would be inappropriate and misleading, as (1) there are cases of cancer where some markers of exhaustion are dissociated from the causes of high antigen load and chronicity [75]; (2) dysfunction sometimes occurs without the expression of traditional markers of exhaustion [71]; and, conversely, (3) traditional markers of exhaustion are sometimes expressed in functional cells [76,77]. This confirms the importance of systematically specifying which meaning of “exhaustion” one has in mind when suggesting targeting exhaustion in a cancer setting.

Crucially, even when focusing on one specific and explicitly described approach to exhaustion, many important difficulties remain. This can be illustrated with the application to cancer of the causal approach of exhaustion. The main suggestion in papers linking cancer and exhaustion has been that the causes characterizing exhaustion in the viral context would also occur in cancer: The chronic exposure of T cells to a high load of tumor antigens would lead to their exhaustion, and, therefore, to the incapacity of these T cells to control tumor growth. This idea led to the hypothesis that poor immune responses to cancer are related to chronic exposure to high levels of tumor “neoantigens” [78,79] (typically seen as a consequence of a high rate of genetic mutations [80,81]). In clinical practice, the expectation has been that a high mutation load would lead to exhaustion, and, therefore, to good responses to anti-PD1 therapies. This is indeed what is seen in some cancer types [82]. For example, neoantigen burden in non-small cell lung cancer is directly correlated with the clinical response to anti-PD-1 therapy [83]. Yet, in a number of other cancer types, the correlation does not hold. Some cancers, such as the renal cell carcinoma, have a better response to anti-PD-1 therapy than the one that would be predicted by the tumor mutational burden. Others, such as colorectal cancer with mismatch repair proficiency, have a response that is worse than the one that would be predicted by the tumor mutational burden [82]. Overall, assuming an overlap between dysfunction on the one hand and chronicity and/or high antigen load on the other when predicting T-cell exhaustion and thus anti-PD1 response would lead to unsuitable therapeutic strategies.

Second, even in the limited number of cases in which there is a satisfactory overlap between the 3 approaches to immunological exhaustion (dysfunction, causes, and markers), focusing on exhaustion remains problematic because it constitutes at best a small subset of the many forms of immunological tolerance to the tumor. There are, in fact, many parameters that help explain the elimination or non-elimination of the tumor by T cells, and each of these parameters can be linked to biomarkers—as suggested by the concept of the “cancer immunogram” [84]. Furthermore, even the focus on CD8 and CD4 T cells is increasingly recognized as too narrow: The nature of the immune response to tumors also depends on innate immune cells such as macrophages and on the tumor micro- and macro-environment [85–88], which all impact T cells’ function and also play T cell-independent roles. Thus, from a clinical point of view, not only must one keep in mind that the concept of exhaustion takes different and often nonoverlapping meanings, but it is also essential to consider that immunological tolerance, be it mediated by “exhaustion” or not, is always dependent on “contextual” factors, some of which can be mechanistically studied and therapeutically manipulated.

Recent work on COVID-19 offers another interesting example of the potential inconsistencies and misunderstandings that can be associated with the concept of exhaustion. CD8+ T cells present an exhausted phenotype in many COVID-19 patients, but the cause involved is not always mentioned [89]. High antigen load seems to be a more likely cause than chronicity since the markers and the dysfunction of CD8+ T cells were observed in as short a period as 7 days postinfection [90,91]. Moreover, such T cells are not always described as dysfunctional [92]. Finally, it is now well known that COVID-19 patients can die from an overstimulation of the immune system, especially via “cytokine storms” [93]. Consequently, misunderstanding “the exhausted profile” as an indiscriminate need for T-cell reinvigoration could lead to clinical disaster for the patient. As with cancer, a productive application of the conceptual framework of exhaustion to COVID-19 will require a precise examination of the 3 approaches and a careful reflection on the full therapeutic consequences of the manipulation of “exhausted” T cells. Fig 4 sums up examples of nonoverlap between the 3 approaches to exhaustion.

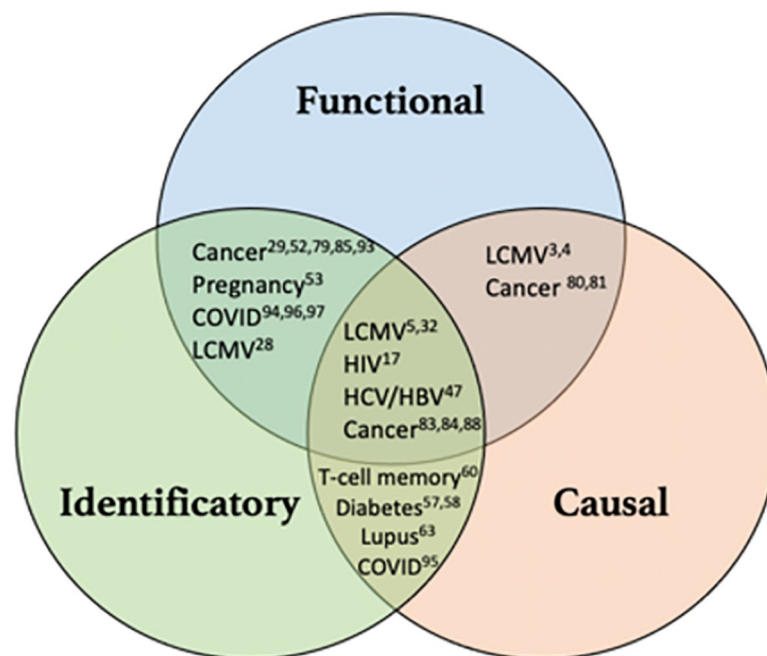


Fig 4. Examples of absence of overlap between the 3 approaches to exhaustion. COVID, Coronavirus Disease; HBV, hepatitis B virus; HCV, hepatitis C virus; LCMV, lymphocytic choriomeningitis virus.

<https://doi.org/10.1371/journal.ppat.1009892.g004>

Conclusions: Exploring new avenues about exhaustion and immunoregulation

From the 1990s to the present day, many immunologists have suggested that exhaustion might in fact reflect an evolutionary conserved process of immunoregulation [5,11], limiting the risk of immunopathology. Indeed, what immunologists have dubbed “exhaustion” to initially describe cells that seem to fail to realize their expected effector functions may well actually be one manifestation of a sometimes beneficial physiological process, preventing excessive immune responses and excessive damages.

One important way to approach this question is to adopt a multilevel perspective. A process that seems dysfunctional at a lower level can in fact be part of a functional regulatory process at a higher level. Typically, a state of exhaustion might be dysfunctional at the cell level while being functional at the tissue or organism level—for instance, if exhaustion helps limit immunopathology. This approach is convergent with a role for exhaustion in the phenomenon of “disease tolerance.” Disease tolerance, a term long used in plant ecology [94] and referring to a reduction of the negative impact of an infection on host fitness without directly affecting the pathogen burden, has been increasingly studied in recent immunology [95,96]. The phenomenon of disease tolerance reflects the fact that, in terms of fitness, it is sometimes better to mitigate the impact of a source of damage rather than eliminate it. Immunological exhaustion has indeed been characterized as one of the causes by which disease tolerance is achieved [97]. Future research will help clarify the concept of exhaustion thanks to a better understanding of its connections with both “disease tolerance” (which has to do with the non-elimination of a source of damage) and “immunological tolerance” (which has to do with the downregulation of an effector immune response).

The perspective presented in this paper opens up original and promising avenues for future research in at least 3 areas:

- i. **Regulation to self:** Future work needs to determine whether immune responses to “self” display some characteristics generally associated with exhaustion (in this case, people will presumably prefer to talk about “regulation”). Recent research has started to explore the role of exhaustion in the prevention of autoimmune diseases [26,98], but more work is needed, and the role of exhaustion must be investigated not only in autoimmune diseases but also in physiological autoimmunity (i.e., physiological responses to “self,” for example, in tissue maintenance and tissue repair). We expect that the experimental inhibition of exhaustion will favor autoimmune diseases and inflammatory diseases, as well as dysregulation of physiological responses to self-constituents.
- ii. **Regulation to “resident nonself”:** Typically, the microbiota. Studies on the microbiota do not even examine the phenomenon of immunological exhaustion. An intriguing hypothesis would be that the experimental inhibition of exhaustion in the gut or on the skin might lead to elimination of some microbes with which the host normally cohabits.
- iii. **Negative consequences of excessive limitation of exhaustion in contexts of the following:**
 - (a) **Infection:** Although limiting exhaustion in some contexts of viral infection is a legitimate objective, we expect that an excessive inhibition of exhaustion could lead to pathological consequences, from immunopathology to the development of autoimmune disorders. The idea of an equilibrium between beneficial and detrimental exhaustion has been present for a long time in the literature on exhaustion and has been specifically

investigated in recent times, but what is needed now is an explicit description of the causes that will insure an adequate equilibrium.

(b) Cancer: There is no doubt that reversing T-cell exhaustion in some cancers can be useful. Yet, as we have seen, the sense given to the “exhaustion” of these cells is not consistently clear, and, in many cases, restoring the functions of these cells does not really amount to suppressing their state of “exhaustion.” Moreover, future research will certainly confirm that the “reinvigoration” of T cells in cancer may lead to detrimental consequences for the host in terms of immunopathology and autoimmune responses, which makes it all the more important to, first, specifically understand the type of immunoregulation (not necessarily reducible to exhaustion) involved in immune responses to cancer, and, second, systematically understand the causes of T-cell responses to cancer in the broader context of the many components of the immunological tumor microenvironment.

(c) Allergies: Allergies are another domain in which, interestingly, exhaustion is almost never investigated. Future studies will have to determine if exhaustion plays a role in the allergic response, and, possibly, in desensitization (where the antigen is chronic but present in low quantities).

Discussing the definition of “exhaustion” is not just a matter of words. It has crucial consequences in experimental and clinical practice.

Acknowledgments

We thank Rafi Ahmed for extremely generous discussions and comments on the paper. Many thanks to Gérard Eberl, Jean-Laurent Casanova, and Rolf Zinkernagel for their comments on the paper and to Victor Appay, Patrick Blanco, Julie Déchanet-Merville, Pierre Merville, Jean-François Moreau, Thierry Schaevebeke, Charlotte Domblides, Guillaume Rebillon, and Eric Vivier for their inputs during the preparation of this project.

References

1. Byers VS, Sercarz EE. The X-Y-Z scheme of immunocyte maturation. IV. The exhaustion of memory cells. *J Exp Med*. 1968; 127:307–25. <https://doi.org/10.1084/jem.127.2.307> PMID: 5635381
2. Byers VS, Sercarz EE. The X-Y-Z scheme of immunocyte maturation. V. Paralysis of memory cells. *J Exp Med*. 1968; 128:715–28. <https://doi.org/10.1084/jem.128.4.715> PMID: 5691983
3. Moskophidis D, Lechner F, Pircher H, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature*. 1993; 362:758–61. <https://doi.org/10.1038/362758a0> PMID: 8469287
4. Gallimore A, Glithero A, Godkin A, Tissot AC, Plückthun A, Elliott T, et al. Induction and Exhaustion of Lymphocytic Choriomeningitis Virus-specific Cytotoxic T Lymphocytes Visualized Using Soluble Tetrameric Major Histocompatibility Complex Class I–Peptide Complexes. *J Exp Med*. 1998; 187:1383–93. <https://doi.org/10.1084/jem.187.9.1383> PMID: 9565631
5. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJD, Suresh M, Altman JD, et al. Viral Immune Evasion Due to Persistence of Activated T Cells Without Effector Function. *J Exp Med*. 1998; 188:2205–13. <https://doi.org/10.1084/jem.188.12.2205> PMID: 9858507
6. Yi JS, Cox MA, Zajac AJ. T-cell exhaustion: characteristics, causes and conversion. *Immunology*. 2010; 129:474–81. <https://doi.org/10.1111/j.1365-2567.2010.03255.x> PMID: 20201977
7. Wu T, Ji Y, Moseman EA, Xu HC, Manglani M, Kirby M, et al. The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci Immunol*. 2016; 1:eaai8593–3. <https://doi.org/10.1126/sciimmunol.aai8593> PMID: 28018990
8. Utzschneider DT, Charmoy M, Chennupati V, Pousse L, Ferreira DP, Calderon-Copete S, et al. T Cell Factor 1-Expressing Memory-like CD8+ T Cells Sustain the Immune Response to Chronic Viral Infections. *Immunity*. 2016; 45:415–27. <https://doi.org/10.1016/j.immuni.2016.07.021> PMID: 27533016

9. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature*. 2016; 537:417–21. <https://doi.org/10.1038/nature19330> PMID: 27501248
10. He R, Hou S, Liu C, Zhang A, Bai Q, Han M, et al. Follicular CXCR5-expressing CD8+ T cells curtail chronic viral infection. *Nature*. 2016; 537:412–6. <https://doi.org/10.1038/nature19317> PMID: 27501245
11. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining T cell exhaustion. *Nat Rev Immunol*. 2019;1–10. <https://doi.org/10.1038/s41577-018-0096-1> PMID: 30482912
12. Rocha B, Grandien A, Freitas AA. Anergy and exhaustion are independent mechanisms of peripheral T cell tolerance. *J Exp Med*. 1995; 181:993–1003. <https://doi.org/10.1084/jem.181.3.993> PMID: 7869056
13. Schietinger A, Greenberg PD. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. *Trends Immunol*. 2014; 35:51–60. <https://doi.org/10.1016/j.it.2013.10.001> PMID: 24210163
14. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell*. 2009; 138:30–50. <https://doi.org/10.1016/j.cell.2009.06.036> PMID: 19596234
15. Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. *J Exp Med*. 2015; 212:1125–37. <https://doi.org/10.1084/jem.20142237> PMID: 26034050
16. Kroy DC, Ciuffreda D, Cooperrider JH, Tomlinson M, Hauck GD, Aneja J, et al. Liver Environment and HCV Replication Affect Human T-Cell Phenotype and Expression of Inhibitory Receptors. *Gastroenterology*. 2014; 146:550–61. <https://doi.org/10.1053/j.gastro.2013.10.022> PMID: 24148617
17. Quigley M, Pereyra F, Nilsson B, Porichis F, Fonseca C, Eichbaum Q, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nat Med*. 2010; 16:1147–51. <https://doi.org/10.1038/nm.2232> PMID: 20890291
18. Im SJ, Ha S-J. Re-defining T-Cell Exhaustion: Subset, Function, and Regulation. *Immune Netw*. 2020; 20:e2. <https://doi.org/10.4110/in.2020.20.e2> PMID: 32158590
19. Khalil DN, Suek N, Campesato LF, Budhu S, Redmond D, Samstein RM, et al. In situ vaccination with defined factors overcomes T cell exhaustion in distant tumors. *J Clin Invest*. 2019; 129:3435–47. <https://doi.org/10.1172/JCI128562> PMID: 31329159
20. Malandro N, Budhu S, Kuhn NF, Liu C, Murphy JT, Cortez C, et al. Clonal Abundance of Tumor-Specific CD4 + T Cells Potentiates Efficacy and Alters Susceptibility to Exhaustion. *Immunity*. 2016; 44:179–93. <https://doi.org/10.1016/j.immuni.2015.12.018> PMID: 26789923
21. Wherry EJ, Blattman JN, Murali-Krishna K, van der R, Ahmed R. Viral Persistence Alters CD8 T-Cell Immunodominance and Tissue Distribution and Results in Distinct Stages of Functional Impairment. *J Virol*. 2003; 77:4911–27. <https://doi.org/10.1128/jvi.77.8.4911-4927.2003> PMID: 12663797
22. Man K, Gabriel SS, Liao Y, Gloury R, Preston S, Henstridge DC, et al. Transcription Factor IRF4 Promotes CD8+ T Cell Exhaustion and Limits the Development of Memory-like T Cells during Chronic Infection. *Immunity*. 2017; 47:1129, e5–41. <https://doi.org/10.1016/j.immuni.2017.11.021> PMID: 29246443
23. Utzschneider DT, Legat A, Fuertes Marraco SA, Carrié L, Luescher I, Speiser DE, et al. T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion. *Nat Immunol*. 2013; 14:603–10. <https://doi.org/10.1038/ni.2606> PMID: 23644506
24. Speiser DE, Utzschneider DT, Oberle SG, Münz C, Romero P, Zehn D. T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? *Nat Rev Immunol*. 2014; 14:768–74. <https://doi.org/10.1038/nri3740> PMID: 25257362
25. Cornberg M, Kenney LL, Chen AT, Kim S-K, Dienes HP, Waggoner S, et al. Clonal Exhaustion as a Mechanism to Protect Against Severe Immunopathology and Death from an Overwhelming CD8 T Cell Response. *Front Immunol*. 2013;4. <https://doi.org/10.3389/fimmu.2013.00004> PMID: 23386848
26. McKinney EF, Lee JC, Jayne DRW, Lyons PA, Smith KGC. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature*. 2015; 523:612–6. <https://doi.org/10.1038/nature14468> PMID: 26123020
27. Bucks CM, Norton JA, Boesteanu AC, Mueller YM, Katsikis PD. Chronic antigen stimulation alone is sufficient to drive CD8+ T cell exhaustion. *J Immunol*. 2009; 182:6697–708. <https://doi.org/10.4049/jimmunol.0800997> PMID: 19454664
28. Khan O, Giles JR, McDonald S, Manne S, Ngiow SF, Patel KP, et al. TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. *Nature*. 2019; 571:211–8. <https://doi.org/10.1038/s41586-019-1325-x> PMID: 31207603
29. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MBA. Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med*. 2006; 12:1301–9. <https://doi.org/10.1038/nm1492> PMID: 17041596

30. Mitchison NA. Induction of Immunological Paralysis in Two Zones of Dosage. *Proc R Soc Lond B Biol Sci.* 1964; 161:275–92. <https://doi.org/10.1098/rspb.1964.0093> PMID: 14224412
31. Bell GI. Mathematical model of clonal selection and antibody production. III. The cellular basis of immunological paralysis. *J Theor Biol.* 1971; 33:379–98. [https://doi.org/10.1016/0022-5193\(71\)90072-5](https://doi.org/10.1016/0022-5193(71)90072-5) PMID: 5167514
32. Mueller SN, Ahmed R. High antigen levels are the cause of T cell exhaustion during chronic viral infection. *Proc Natl Acad Sci U S A.* 2009; 106:8623–8. <https://doi.org/10.1073/pnas.0809818106> PMID: 19433785
33. Tinoco R, Alcalde V, Yang Y, Sauer K, Zuniga EI. Cell-Intrinsic Transforming Growth Factor- β Signaling Mediates Virus-Specific CD8+ T Cell Deletion and Viral Persistence In Vivo. *Immunity.* 2009; 31:145–57. <https://doi.org/10.1016/j.immuni.2009.06.015> PMID: 19604493
34. Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. *J Exp Med.* 2006; 203:2461–72. <https://doi.org/10.1084/jem.20061462> PMID: 17030951
35. Yi JS, Du M, Zajac AJ. A Vital Role for Interleukin-21 in the Control of a Chronic Viral Infection. *Science.* 2009; 324:1572–6. <https://doi.org/10.1126/science.1175194> PMID: 19443735
36. Penaloza-MacMaster P, Kamphorst AO, Wieland A, Araki K, Iyer SS, West EE, et al. Interplay between regulatory T cells and PD-1 in modulating T cell exhaustion and viral control during chronic LCMV infection. *J Exp Med.* 2014; 211:1905–18. <https://doi.org/10.1084/jem.20132577> PMID: 25113973
37. Park HJ, Park JS, Jeong YH, Son J, Ban YH, Lee B-H, et al. PD-1 Upregulated on Regulatory T Cells during Chronic Virus Infection Enhances the Suppression of CD8 + T Cell Immune Response via the Interaction with PD-L1 Expressed on CD8 + T Cells. *J Immunol.* 2015; 194:5801–11. <https://doi.org/10.4049/jimmunol.1401936> PMID: 25934860
38. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. *Cell Death Dis.* 2015; 6:e1792. <https://doi.org/10.1038/cddis.2015.162> PMID: 26086965
39. Scott AC, Dündar F, Zumbo P, Chandran SS, Klebanoff CA, Shakiba M, et al. TOX is a critical regulator of tumour-specific T cell differentiation. *Nature.* 2019; 571:270–4. <https://doi.org/10.1038/s41586-019-1324-y> PMID: 31207604
40. Araki K, Youngblood B, Ahmed R. Programmed Cell Death 1-Directed Immunotherapy for Enhancing T-Cell Function. *Cold Spring Harb Symp Quant Biol.* 2013; 78:239–47. <https://doi.org/10.1101/sqb.2013.78.019869> PMID: 25028401
41. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. *Annu Rev Immunol.* 2019; 37:457–95. <https://doi.org/10.1146/annurev-immunol-041015-055318> PMID: 30676822
42. Paley MA, Kroy DC, Odorizzi PM, Johnnidis JB, Dolfi DV, Barnett BE, et al. Progenitor and Terminal Subsets of CD8+ T Cells Cooperate to Contain Chronic Viral Infection. *Science.* 2012; 338:1220–5. <https://doi.org/10.1126/science.1229620> PMID: 23197535
43. Chennupati V, Held W. Feeling Exhausted? Tuning Irf4 Energizes Dysfunctional T Cells. *Immunity.* 2017; 47:1009–11. <https://doi.org/10.1016/j.immuni.2017.11.028> PMID: 29262341
44. Alvisi G, Brummelman J, Puccio S, Mazza EMC, Tomada EP, Losurdo A, et al. IRF4 instructs effector Treg differentiation and immune suppression in human cancer. *J Clin Invest.* 2020; 130:3137–50. <https://doi.org/10.1172/JCI130426> PMID: 32125291
45. Bengsch B, Johnson AL, Kurachi M, Odorizzi PM, Pauken KE, Attanasio J, et al. Bioenergetic Insufficiencies Due to Metabolic Alterations Regulated by the Inhibitory Receptor PD-1 Are an Early Driver of CD8 + T Cell Exhaustion. *Immunity.* 2016; 45:358–73. <https://doi.org/10.1016/j.immuni.2016.07.008> PMID: 27496729
46. Chang C-H, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell.* 2013; 153:1239–51. <https://doi.org/10.1016/j.cell.2013.05.016> PMID: 23746840
47. Kurtschiev PD, Raziorrouh B, Schraut W, Backmund M, Wächtler M, Wendtner C-M, et al. Dysfunctional CD8+ T cells in hepatitis B and C are characterized by a lack of antigen-specific T-bet induction. *J Exp Med.* 2014; 211:2047–59. <https://doi.org/10.1084/jem.20131333> PMID: 25225458
48. Klenerman P. The (gradual) rise of memory inflation. *Immunol Rev.* 2018; 283:99–112. <https://doi.org/10.1111/imr.12653> PMID: 29664577
49. Ochel A, Cebula M, Riehn M, Hillebrand U, Lipps C, Schirmbeck R, et al. Effective intrahepatic CD8+ T-cell immune responses are induced by low but not high numbers of antigen-expressing hepatocytes. *Cell Mol Immunol.* 2016; 13:805–15. <https://doi.org/10.1038/cmi.2015.80> PMID: 26412123

50. Han S, Asoyan A, Rabenstein H, Nakano N, Obst R. Role of antigen persistence and dose for CD4+ T-cell exhaustion and recovery. *Proc Natl Acad Sci U S A*. 2010; 107:20453–8. <https://doi.org/10.1073/pnas.1008437107> PMID: 21059929
51. Mohme M, Schliifke S, Maire CL, Runger A, Glau L, Mende KC, et al. Immunophenotyping of Newly Diagnosed and Recurrent Glioblastoma Defines Distinct Immune Exhaustion Profiles in Peripheral and Tumor-infiltrating Lymphocytes. *Clin Cancer Res*. 2018; 24:4187–200. <https://doi.org/10.1158/1078-0432.CCR-17-2617> PMID: 29444930
52. Kinder JM, Turner LH, Stelzer IA, Miller-Handley H, Burg A, Shao T-Y, et al. CD8+ T Cell Functional Exhaustion Overrides Pregnancy-Induced Fetal Antigen Alloimmunization. *Cell Rep*. 2020; 31:107784. <https://doi.org/10.1016/j.celrep.2020.107784> PMID: 32579916
53. Riley JL. PD-1 signaling in primary T cells. *Immunol Rev*. 2009; 229:114–25. <https://doi.org/10.1111/j.1600-065X.2009.00767.x> PMID: 19426218
54. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol*. 2009; 10:29–37. <https://doi.org/10.1038/ni.1679> PMID: 19043418
55. Ansari MJ, Salama AD, Chitnis T, Smith RN, Yagita H, Akiba H, et al. The Programmed Death-1 (PD-1) Pathway Regulates Autoimmune Diabetes in Nonobese Diabetic (NOD) Mice. *J Exp Med*. 2003; 198:63–9. <https://doi.org/10.1084/jem.20022125> PMID: 12847137
56. Youssef N, Noureldein M, Daoud G, Eid AA. Immune checkpoint inhibitors and diabetes: Mechanisms and predictors. *Diabetes Metab*. 2020;S1262363620301415. <https://doi.org/10.1016/j.diabet.2020.09.003> PMID: 33010422
57. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and Its Ligands in Tolerance and Immunity. *Annu Rev Immunol*. 2008; 26:677–704. <https://doi.org/10.1146/annurev.immunol.26.021607.090331> PMID: 18173375
58. Pauken KE, Godec J, Odorizzi PM, Brown KE, Yates KB, Ngiow SF, et al. The PD-1 Pathway Regulates Development and Function of Memory CD8+ T Cells following Respiratory Viral Infection. *Cell Rep*. 2020; 31:107827. <https://doi.org/10.1016/j.celrep.2020.107827> PMID: 32610128
59. Frebel H, Nindl V, Schuepbach RA, Braunschweiler T, Richter K, Vogel J, et al. Programmed death 1 protects from fatal circulatory failure during systemic virus infection of mice. *J Exp Med*. 2012; 209:2485–99. <https://doi.org/10.1084/jem.20121015> PMID: 23230000
60. Tilstra JS, Avery L, Menk AV, Gordon RA, Smita S, Kane LP, et al. Kidney-infiltrating T cells in murine lupus nephritis are metabolically and functionally exhausted. *J Clin Invest*. 2018; 128:4884–97. <https://doi.org/10.1172/JCI120859> PMID: 30130253
61. Zezza M, Kosinski C, Mekoguem C, Marino L, Chtioui H, Pitteloud N, et al. Combined immune checkpoint inhibitor therapy with nivolumab and ipilimumab causing acute-onset type 1 diabetes mellitus following a single administration: two case reports. *BMC Endocr Disord*. 2019; 19:144. <https://doi.org/10.1186/s12902-019-0467-z> PMID: 31870373
62. Cui W, Kaech SM. Generation of effector CD8+ T cells and their conversion to memory T cells: Effector and memory T-cell differentiation. *Immunol Rev*. 2010; 236:151–66. <https://doi.org/10.1111/j.1600-065X.2010.00926.x> PMID: 20636815
63. Alfei F, Kanev K, Hofmann M, Wu M, Ghoneim HE, Roelli P, et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature*. 2019; 571:265–9. <https://doi.org/10.1038/s41586-019-1326-9> PMID: 31207605
64. Wu J, Zhang H, Shi X, Xiao X, Fan Y, Minze LJ, et al. Ablation of Transcription Factor IRF4 Promotes Transplant Acceptance by Driving Allogeneic CD4+ T Cell Dysfunction. *Immunity*. 2017; 47:1114, e6–28. <https://doi.org/10.1016/j.immuni.2017.11.003> PMID: 29221730
65. Doedens AL, Phan AT, Stradner MH, Fujimoto JK, Nguyen JV, Yang E, et al. Hypoxia-inducible factors enhance the effector responses of CD8+ T cells to persistent antigen. *Nat Immunol*. 2013; 14:1173–82. <https://doi.org/10.1038/ni.2714> PMID: 24076634
66. Lesokhin AM, Callahan MK, Postow MA, Wolchok JD. On being less tolerant: Enhanced cancer immunosurveillance enabled by targeting checkpoints and agonists of T cell activation. *Sci Transl Med*. 2015; 7:280sr1–1. <https://doi.org/10.1126/scitranslmed.3010274> PMID: 25810313
67. Sharma P, Allison JP. Dissecting the mechanisms of immune checkpoint therapy. *Nat Rev Immunol*. 2020; 20:75–6. <https://doi.org/10.1038/s41577-020-0275-8> PMID: 31925406
68. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol*. 2015; 36:265–76. <https://doi.org/10.1016/j.it.2015.02.008> PMID: 25797516
69. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, et al. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science*. 2016; 354:1160–5. <https://doi.org/10.1126/science.aaf2807> PMID: 27789795

70. Pradeu T, Vivier E. The discontinuity theory of immunity. *Sci Immunol*. 2016; 1:aag0479–9. <https://doi.org/10.1126/sciimmunol.aag0479> PMID: 28239677
71. Schietinger A, Philip M, Krisnawan VE, Chiu EY, Delrow JJ, Basom RS, et al. Tumor-Specific T Cell Dysfunction Is a Dynamic Antigen-Driven Differentiation Program Initiated Early during Tumorigenesis. *Immunity*. 2016; 45:389–401. <https://doi.org/10.1016/j.immuni.2016.07.011> PMID: 27521269
72. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature*. 2017; 545:60–5. <https://doi.org/10.1038/nature22079> PMID: 28397821
73. Mumprecht S, Schürch C, Schwaller J, Solenthaler M, Ochsenbein AF. Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression. *Blood*. 2009; 114:1528–36. <https://doi.org/10.1182/blood-2008-09-179697> PMID: 19420358
74. Baitsch L, Baumgaertner P, Devèvre E, Raghav SK, Legat A, Barba L, et al. Exhaustion of tumor-specific CD8+ T cells in metastases from melanoma patients. *J Clin Invest*. 2011; 121:2350–60. <https://doi.org/10.1172/JCI46102> PMID: 21555851
75. Kaiser AD, Schuster K, Gadiot J, Borkner L, Daebritz H, Schmitt C, et al. Reduced tumor-antigen density leads to PD-1/PD-L1-mediated impairment of partially exhausted CD8+ T cells. *Eur J Immunol*. 2012; 42:662–71. <https://doi.org/10.1002/eji.201141931> PMID: 22144176
76. Legat A, Speiser DE, Pircher H, Zehn D, Fuertes Marraco SA. Inhibitory Receptor Expression Depends More Dominantly on Differentiation and Activation than “Exhaustion” of Human CD8 T Cells. *Front Immunol*. 2013; 4. <https://doi.org/10.3389/fimmu.2013.00455> PMID: 24391639
77. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood*. 2013; 121:1612–21. <https://doi.org/10.1182/blood-2012-09-457531> PMID: 23247726
78. Schumacher TN, Scheper W, Kvistborg P. Cancer Neoantigens. *Annu Rev Immunol*. 2019; 37:173–200. <https://doi.org/10.1146/annurev-immunol-042617-053402> PMID: 30550719
79. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015; 348:69–74. <https://doi.org/10.1126/science.aaa4971> PMID: 25838375
80. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature*. 2013; 500:415–21. <https://doi.org/10.1038/nature12477> PMID: 23945592
81. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Ng AWT, Wu Y, et al. The repertoire of mutational signatures in human cancer. *Nature*. 2020; 578:94–101. <https://doi.org/10.1038/s41586-020-1943-3> PMID: 32025018
82. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med*. 2017; 377:2500–1. <https://doi.org/10.1056/NEJMc1713444> PMID: 29262275
83. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015; 348:124–8. <https://doi.org/10.1126/science.aaa1348> PMID: 25765070
84. Blank CU, Haanen JB, Ribas A, Schumacher TN. The “cancer immunogram.”. *Science*. 2016; 352:658–60. <https://doi.org/10.1126/science.aaf2834> PMID: 27151852
85. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, et al. Differences in Tumor Microenvironment Dictate T Helper Lineage Polarization and Response to Immune Checkpoint Therapy. *Cell*. 2019; 179:1177, e13–90. <https://doi.org/10.1016/j.cell.2019.10.029> PMID: 31730856
86. Laplane L, Duluc D, Bikfalvi A, Larmonier N, Pradeu T. Beyond the tumour microenvironment. *Int J Cancer*. 2019; 145:2611–8. <https://doi.org/10.1002/ijc.32343> PMID: 30989643
87. Pitt JM, Vétizou M, Daillère R, Roberti MP, Yamazaki T, Routy B, et al. Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors. *Immunity*. 2016; 44:1255–69. <https://doi.org/10.1016/j.immuni.2016.06.001> PMID: 27332730
88. Duan J, Wang Y, Jiao S. Checkpoint blockade-based immunotherapy in the context of tumor microenvironment: Opportunities and challenges. *Cancer Med*. 2018; 7:4517–29. <https://doi.org/10.1002/cam4.1722> PMID: 30088347
89. Mahmoudi S, Rezaei M, Mansouri N, Marjani M, Mansouri D. Immunologic Features in Coronavirus Disease 2019: Functional Exhaustion of T Cells and Cytokine Storm. *J Clin Immunol*. 2020; 40:974–6. <https://doi.org/10.1007/s10875-020-00824-4> PMID: 32648027
90. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science*. 2020; 369: eabc8511. <https://doi.org/10.1126/science.abc8511> PMID: 32669297
91. Zheng H-Y, Zhang M, Yang C-X, Zhang N, Wang X-C, Yang X-P, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19

- patients. *Cell Mol Immunol*. 2020; 17:541–3. <https://doi.org/10.1038/s41423-020-0401-3> PMID: [32203186](https://pubmed.ncbi.nlm.nih.gov/32203186/)
92. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun*. 2020; 11:3434. <https://doi.org/10.1038/s41467-020-17292-4> PMID: [32632085](https://pubmed.ncbi.nlm.nih.gov/32632085/)
 93. Chen Z, John WE. T cell responses in patients with COVID-19. *Nat Rev Immunol*. 2020; 20:529–36. <https://doi.org/10.1038/s41577-020-0402-6> PMID: [32728222](https://pubmed.ncbi.nlm.nih.gov/32728222/)
 94. Schafer JF. Tolerance to Plant Disease. *Annu Rev Phytopathol*. 1971; 9:235–52. <https://doi.org/10.1146/annurev.py.09.090171.001315>
 95. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. *Science*. 2012; 335:936–41. <https://doi.org/10.1126/science.1214935> PMID: [22363001](https://pubmed.ncbi.nlm.nih.gov/22363001/)
 96. Martins R, Carlos AR, Braza F, Thompson JA, Bastos-Amador P, Ramos S, et al. Disease Tolerance as an Inherent Component of Immunity. *Annu Rev Immunol*. 2019; 37:405–37. <https://doi.org/10.1146/annurev-immunol-042718-041739> PMID: [30673535](https://pubmed.ncbi.nlm.nih.gov/30673535/)
 97. McCarville J, Ayres J. Disease tolerance: concept and mechanisms. *Curr Opin Immunol*. 2018; 50:88–93. <https://doi.org/10.1016/j.coi.2017.12.003> PMID: [29253642](https://pubmed.ncbi.nlm.nih.gov/29253642/)
 98. McKinney EF, Smith KG. T cell exhaustion and immune-mediated disease—the potential for therapeutic exhaustion. *Curr Opin Immunol*. 2016; 43:74–80. <https://doi.org/10.1016/j.coi.2016.09.005> PMID: [27744240](https://pubmed.ncbi.nlm.nih.gov/27744240/)