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Review

Leprosy as a model to understand cancer immunosurveillance and T cell anergy

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ABSTRACT

Leprosy is a disease caused by *Mycobacterium leprae* that presents on a spectrum of both clinical manifestations and T cell response. On one end of this spectrum, tuberculoid leprosy is a well-controlled disease, characterized by a cell-mediated immunity and immunosurveillance. On the opposite end of the spectrum, lepromatous leprosy is characterized by *M. leprae* proliferation and T cell anergy. Similar to progressive tumor cells, *M. leprae* escapes immunosurveillance in more severe forms of leprosy. The mechanisms by which *M. leprae* is able to evade the host immune response involve many, including the alterations of lipid droplets, microRNA, and Schwann cells, and involve the regulation of immune regulators, such as the negative checkpoint regulators CTLA-4, programmed death 1, and V-domain Ig suppressor of T cell activation—important targets in today's cancer immunotherapies. The means by which tumor cells become able to escape immunosurveillance through negative checkpoint regulators are evidenced by the successes of treatments, such as nivolumab and ipilimumab. Many parallels can be drawn between the immune responses seen in leprosy and cancer. Therefore, the understanding of how *M. leprae* encourages immune escape during proliferative disease states has potential to add to our understanding of cancer immunotherapy. *J. Leukoc. Biol.* 100: 47–54; 2016.

Introduction

The latest progress in cancer therapies has been attributed to immunotherapy. In fact, immunotherapy was recognized as the "the breakthrough of the year for 2013" by the editors of *Science* [1]. The idea to use the host's own immune defenses against malignancies is not new, but only recently have technology and advanced understanding allowed for progress in the field of immunotherapy.

Abbreviations: Bcl-2 = B cell lymphoma 2, BT = borderline tuberculoid, Cbl-b = an E3 ubiquitin-protein ligase that also functions as a negative regulator of T cell activation, CD = cluster of differentiation, ENL = erythema nodosum leprosum, FDA = U.S. Food and Drug Administration, Foxp3 = forkhead box p3, iT_{reg} = inducible regulatory T cell, LL = lepromatous leprosy, miR = microRNA, miRNA = microRNA, NCR = negative checkpoint regulator;

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The most recent advances are based on the concept that NCRs are in place to restrict T cell response. Release of this restriction using targeting antibodies is at the core of immunotherapy, with treatments focusing on blocking NCRs, such as PD-1 and CTLA-4. Recently, VISTA was added to this group of NCRs. Preclinical studies have shown it to be a promising target for T cell-mediated cancer immunotherapy [2, 3]. These molecules are members of the B7 family of membrane proteins and control whether antigen stimulation of TCRs and the corresponding costimulation will lead to T cell activation or anergy. The best-studied example of a costimulatory molecule required for T cell activation is CD28, which is activated upon binding to the ligand CD80 or CD86 [4].

Whereas significant progress has been made in the field of cancer immunotherapy, this mode of therapeutics is still far from ideal. Greater understanding of cancer immunotherapy may require going back in history. Leprosy, a disease of ancient times, caused by *M. leprae*, was the first disease to be classified according to the adaptive T cell response. Leprosy as a model has provided a great deal of insight into the nature of T cells and can be used to understand how disease processes, such as cancer, modulate the host immune system. The understanding of how *M. leprae* encourages immune escape during proliferative disease states has potential to add to our understanding of cancer immunotherapy.

LEPROSY AS A MODEL

Whereas the course of disease and etiology is different between infections and cancers, infectious diseases have been at the basis of our understanding of immune response and provide excellent models to understand both the effectiveness and shortcomings of the host immune response to tumors [5].

Leprosy is caused by the intracellular pathogen *M. leprae*. This ancient disease is often forgotten by the layperson in the developing world. Nonetheless, the disease is still prevalent in the developing world and poses a significant health and economic burden on these societies [6]. Whereas drugs are available for the treatment of leprosy, it often leaves cured patients with permanent neurologic deficits and significant tissue damage. Even though the disease is not one that receives much attention from the clinics of

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nonendemic countries, because of the variable presentation of the disease, it provides valuable insights into understanding immune reactions in diseases not limited to infectious diseases (7–9).

The disease presents on a spectrum of clinical manifestations that can be likened to the severity of malignancy and potential for invasion seen with cancer. At the ends of the spectrum are TL and LL. Patients with TLare able to resist the growth of *M. leprae*, thereby limiting the number of lesions in tissue, while nerve damage may still be present. On the other end of the spectrum, patients with LL present with widespread, uncontrolled infection with multiple skin lesions. Whereas this article will focus on these 2 extremes on the leprosy spectrum, the severity of disease can be broken down further into 5 categories: tuberculoid (paucibacillary), BT, midborderline, borderline lepromatous, and lepromatous (multibacillary) [10].

The anergy, or the inactivation of effector T cell response, toward tumor cells can be compared with the anergy seen in multibacillary LL, in which *M. leprae* is able to evade a cell-mediated response. Normally, an effective immune response to *M. leprae* requires cell-mediated activation of T cells, first through signaling TCR and antigen-induced MHC on APCs and a second, signal through costimulation between the T cell and the APC. Perhaps the most important category of professional APCs in leprosy includes dendritic cells, which digest *M. leprae* by phagocytosis or receptor-mediated endocytosis before displaying antigens bound to MHC class II molecules on the cell surface. The ligands CD80 and CD86 are important molecules expressed on APCs that have a crucial role in this costimulatory signal. These molecules have a high affinity for binding with costimulatory molecule CD28 on the T cell. CD80 also has high affinity for binding with the immune checkpoint CTLA-4 on T cells that plays an important role in T cell anergy (discussed later) [11].

The TL and LL divergence can also be characterized by a spectrum between cell-mediated immunity and humoral immunity. Unlike TL, there is a greater antibody response in patients with LL, which has led many to believe that a humoral response does improve host defense against the pathogen [12]. On the other hand, the $CD4^+$ T cell predominates in TL, leading to a Th1 cytokine profile, including IFN-γ, IL-2, and lymphotoxin. $CD8^+$ T cells predominate in LL with a Th2 and IL-4-, IL-5-, and IL-10-dominated cytokine profile [12]. This binary view that Th1 and Th2 are found at opposite ends of the spectrum, however, is a relationship that may oversimplify the pathophysiology of the disease, especially as some studies have failed to demonstrate a clear Th1 and Th2 switch between TL and LL [13, 14]. Instead, TL and LL can be distinguished based on a spectrum of T cell inactivation, or anergy.

LL is often characterized as having a dysfunctional or impaired T cell response with antigen-specific anergy, whereas the mounted Th1 cell seen in TL is robust. In vitro experiments have shown that PBMCs from patients with LL have a defective CD86 expression, which may lead to the anergic phenotype seen with these patients [15]. Furthermore, patients with LL show fewer positive costimulatory CD28 and CD86 markers on T cells. In fact, 1 study found that $CD8^+$ LL clones were found to be $CD28^-$, while still coexpressing CD80 and CD86 [16]. Patients with TL, on the other hand, show an increase in CTLA-4 and PD-1 that is thought to modulate an active immune response [15]. Studies looking at the expression of CD86 and CD80 in non-T cell lines show corresponding findings of the expression of costimulatory molecules comparing patients with TL with patients with LL patients. $CD14^+$ monocytes from patients with LL were found to have significantly lower expression of CD86 compared with patients with TL. This same group found that CD86, but not CD80, was differentially more crucial in the activation of monocytes by leprosy antigens [15].

IMMUNOEDITING AND ESCAPE OF IMMUNOSURVEILLANCE BY LEPROSY AND CANCERS

Tumor cells and *M. leprae* may share a common mechanism of immunoediting to allow for their survival by avoiding destruction by the host with the end result of proliferation and invasion. The concept of immunoediting is one that is applied to cancer immunity and describes the changes in the immunogenicity of pathogenic cells as a result of the anti-tumor response of the immune system, resulting in the emergence of immune-resistant variants. The "three Es" are used to describe different phases of immunoediting: elimination, equilibrium, and escape [17]. We will use this framework of immunoediting to describe the clinical manifestations of leprosy: elimination to describe TL when *M. leprae* proliferation is limited and immune escape to describe LL when pathogen proliferation is rampant (Fig. 1).

During the "elimination" phase, or immunosurveillance, immune effector cells, such as NK cells, with the help of dendritic and $CD4^+$ T cells, are able to recognize and eliminate tumor cells. Therefore, malignant tumor cells are able to escape immunosurveillance. The elimination phase includes innate and adaptive immune responses against tumor cells. For the innate immune response, several effector cells, such as NK cells and T cells, are activated by the inflammatory cytokines that are released by the growing tumor cells, macrophages, and stromal cells surrounding the tumor cells. The recruited NK cells and macrophages produce IL-12 and IFN-γ that kill tumor cells through cytotoxic mechanisms, such as with perforin, TRAILs, and ROS.

During the "equilibrium" phase, tumor cells that have escaped the elimination phase have a nonimmunogenic phenotype selected for growth. It is the longest of the 3 processes in immunoediting and may occur over a period of many years. During this time of Darwinian selection, new tumor cell variants emerge with various mutations that further increase overall resistance to immune attack. During the preceding "escape" phase, tumor cell variants selected for growth during the equilibrium phase have escaped the host's immune defenses with various genetic and epigenetic changes, conferring further resistance to immune detection and destruction [18, 19].

The tumor antigens and immune mechanisms that underlie immunoediting are poorly understood. Two studies showed that immunoediting can be triggered by strongly immunogenic tumor antigens that involve the $CD8^+$ T cell-mediated clearance of antigenic tumor cells. Tumor-specific mutant proteins

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PD-1 = programmed cell death protein 1, PD-L1 = programmed cell death protein ligand 1, PPAR = peroxisome proliferator-activated receptor, pSLC = progenitor/stem-like cell, RORc = retinoic acid receptor-related orphan receptor gamma, ROS = reactive oxygen species, TL = tuberculoid leprosy, T_{reg} = regulatory T cell, VISTA = V-domain Ig suppressor of T cell activation

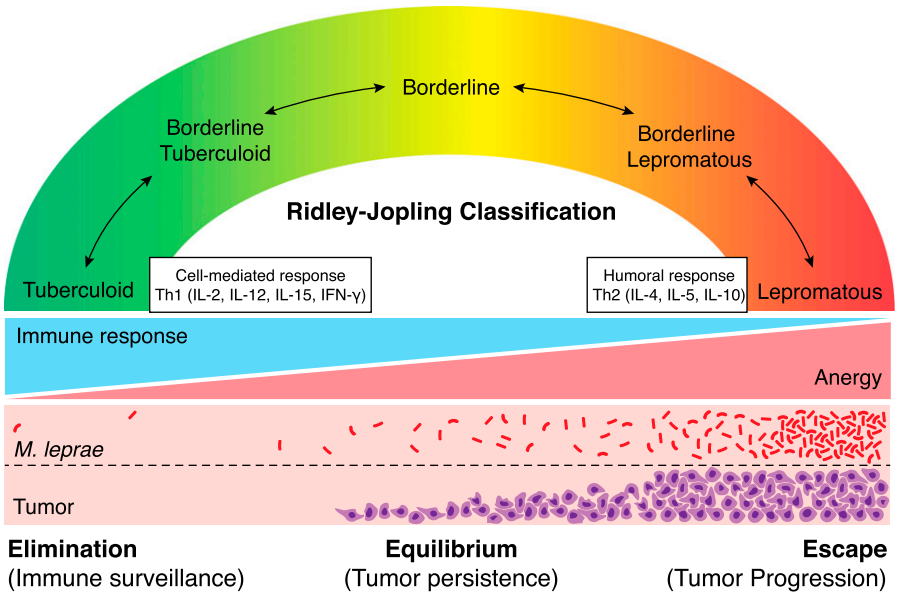

Figure 1. The anergy of leprosy as a paradigm for cancer anergy and immunosurveillance. Leprosy presents as a spectrum of manifestations that is well characterized by the Ridley-Jopling classification. On one end of the leprosy spectrum is TL, which is an effective immune response and controlled *M. leprae* population. TL is often characterized as a cell-mediated response with a Th1 cytokine profile. The other end of the spectrum is LL, which is characterized by T cell anergy, proliferation of *M. leprae*, and a Th2 cytokine profile [89]. Parallels can be drawn from leprosy as a model to understand better the spectrum of immunosurveillance and immunoediting of tumor cells, as characterized by the "3 Es": elimination, equilibrium, and escape [18, 22, 90].

(neo-antigens) might be the key tumor rejection antigens, examples of which include 2 mutant forms of cyclin-dependent kinase 4, R24L and R24C, found in human melanoma [20]. Tumor escape mechanisms have focused mainly on mutations of immune and apoptotic pathway genes. However, data suggest that epigenetic and miRNA silencing in cancer may be as frequent a cause of gene inactivation as mutations [23].

ALTERATIONS OF LIPIDS AND NERVE CELLS

One mechanism by which infection in LL is thought to evade the host immune response involves lipid bodies. LL exhibits the accumulation of foamy macrophages; however, the origin and nature of these lipids in leprosy remain unclear. Macrophages in LL dermal lesions are highly positive for adipose differentiation-related protein, suggesting that their foamy aspect is, at least in part, derived from lipid droplet accumulation [24]. It is speculated that lipid storage in LL plays a role in leprosy pathogenesis by facilitating bacterial persistence by at least 2 different ways. First, foamy macrophages in LL lesions are probably catalytically active sites of PGE_2 synthesis, favoring the inhibition of macrophage bactericidal activities and subversion of the immune response. Second, lipids constitute an important nutritional source for mycobacterial persistence in the host. Analysis of the leprosy proteome showed the presence of the fatty acid β-oxidation and glyoxylate cycle enzymes, reinforcing the idea that fatty acids, rather than carbohydrates, are more likely to be the dominant carbon substrate used by *M. leprae* during infection [25].

Alterations of lipid metabolism and lipid-related transcription factors and enzymes are known mechanisms in tumor cell survival and immune escape. PPARs are induced in response to nutritional deficiencies and environmental insults. PPARα specifically is important to lipid metabolism in the liver, yet its overexpression is linked to the induction of some tumor cells, such as hepatic tumors, leading to the generation of ROS through the oxidation of fatty acid chains. PPARα can lead to carcinogenetic phenotypes via the suppression of apoptosis and the promotion of dysfunctional cell proliferation [26]. Similar findings have also been demonstrated with peroxidases, such as in the high expression of peroxiredoxins and thioredoxins found in human breast carcinomas [27].

Whereas lipids can be used by tumor cells to defend themselves against the host immune system, the reverse is also true: host lipids can play an important role in immunosurveillance against tumor cells. One example by which this occurs is in CD1-restricted T cells that recognize self-lipid antigens presented on a subset of CD1 molecules, CD1c. The self-lipid antigen, methyl-lysophosphatidic acids, accumulates in primary acute myeloid and B cell acute leukemias. These endogenous lipids are displayed on $CD1c^+$ leukemia cells promoting an autoreactive response from T cells [28]. This mechanism of lipid-antigen presentation parallels what is seen clinically in patients with patients, as skin from patients with TL is associated with a strong induction of all 3 CD1 proteins (a, b, and c), whereas patients with LL lack induction of CD1 proteins on $CD83^+$ dendritic cells [29].

M. leprae also reprograms adult Schwann cells, its preferred host niche, to a stage of pSLCs of mesenchymal traits by down-regulating Schwann cell lineage- and differentiation-associated genes and up-regulating genes of mesoderm development [30]. Reprogramming, accompanied by epigenetic changes, renders infected cells highly plastic, migratory, and immunomodulatory. pSLC promotes bacterial spread by 2 distinct mechanisms: direct differentiation to mesenchymal tissues, including skeletal and smooth muscles, and by forming granuloma-like structures that subsequently release bacteria-laden macrophages. Leprosy uses adult Schwann cells as the primary nonimmune tissue cells for initial colonization and converts infected cells to pSLCs with the capacity to produce chemoattractant and trophic factors, which in turn, promote macrophage recruitment, bacterial transfer, and survival of infected macrophages [30]. Similar induction of nerve cells to express stem-like cell properties has been shown, as in 1 demonstration of high

podocalyxin-like protein expression associated with high-grade glioblastoma multiforme and poor clinical outcomes [31].

miRNA

The understanding of how *M. leprae* modulates host gene expression involves discussion about miRNAs, which code for just 1% of the genomic transcript in mammals yet are predicted to control the activity of >60% of genes and, therefore, have a significant role in the pathogenesis of diseases. Their ubiquity makes them potential targets for treatment. Several miRNAs have been shown to alter the activity of APCs, NK cells, T cell differentiation, and effector capacity in mycobacterial infections [32, 33]. In a case-control study that enrolled 1098 individuals, single nucleotide polymorphisms located in predicted miR-146a was a unique gene associated with increased risk of leprosy and modulation of TNF levels [34]. The pathogenesis of tuberculosis, a disease caused by a species of *Mycobacterium*, has been linked to several miRNAs, including miR-29, -147, -21, -99b, -125b, -155, -144, -223, and -424.

As an example, miR-29 leads to the inhibition of macrophage digestion. The same miRNA is also important in the regulation of apoptosis via the binding of targets as Bcl-2 and myeloid cell leukemia-1 [32]. miR-21 was shown to be specific to LL and targeted key players in the innate immune response, including the down-regulation of TLR2/1, the inhibition of vitamin D-dependent antimicrobial peptides, and the up-regulation of IL-10 [35]. This finding in leprosy has potential to enhance our understanding of how miRNA, such as miR-21, responds in cancers, such as those in the kidneys, colon, and breasts, for which miR-21 is showing promise as both a biomarker and target for disease [36]. miR-92a is another such example that has potential to be used as a biomarker for colorectal cancer [37].

T CELL EXHAUSTION

M. leprae and tumor cells modulate their surrounding physical environment to favor survival, but a true testament to their ability to proliferate and survive is their ability to render the host adaptive immune response dysfunctional and ineffective. T cell anergy is an important factor in tumor progression, especially in the face of an imbalance between stimulatory factors and inhibitory factors. T cells in the tumor microenvironment present antigen with a suboptimal level of CD28 costimulation, resulting in an anergic phenotype. One of the first abilities of T cells to be lost is the ability of antigen-specific T cells to proliferate and produce IL-2 [38, 39]. The chronic stimulation on the immune system by an infection or tumor is known to cause a phenomenon known as T cell exhaustion, associated with a significant reduction in IL-2, IFN-γ, and TNF-α and an overall decrease in the ability to eliminate infection or tumor.

T cell exhaustion is an intrinsic defect in the effector activities of T cells rather than a result of the effects of extrinsic inhibitory signals, such as through inhibitory factors T_{regs} or IL-10 [39]. These intrinsic defects correlate with a down-regulation of type 1 cytokine secretions and an increase in the expression of NCRs, such as PD-1, CTLA-4, T cell Ig and mucin domain-containing molecule 3, lymphocyte activation gene 3, and CD244 [40–44]. Loss of costimulatory signals and the high expression of these inhibitor factors have been shown in a number of cancers, including melanoma and cancers of the lung, breast, head, and neck [45–47]. Chronic inflammation linked to T cell exhaustion is also associated with the presence of a Th2 cytokine profile in the tumor microenvironment. These changes mirror the changes seen in progressive LL disease with its Th2 cytokine profile that includes IL-10 and IFN-β [48].

An emerging topic in this field is the role of IL-9 in the role of T cell exhaustion in the tumor and infection environments. IL-9 is a cytokine produced by Th9 cells that has complex properties. Whereas it has been shown to inhibit the progression of melanoma and carcinomas of the lung, it also has antiapoptotic properties linked to growth of other malignancies, such as T cell lymphoma and Bcl in mice [49, 50]. In the melanoma mouse model, Th9 and IL-9 production is linked to the promotion of a robust $CD8^+$ T cell-mediated antitumor response.

Although IL-9 is linked to an impaired Th1 immune response in patients infected with *M. tuberculosis*, possibly contributing to disease [51], IL-9 in *M. Leprae*-induced cytotoxic T cells, inducible by IFN-γ, was able to reverse the inhibitory action of IL-10 and IL-13. Furthermore, IL-9 improved the lytic activity stimulated by IL-2 and IL-6 in patients with leprosy. Therefore, the selective activation of IL-9 may play a unique role in mitigating the effects of T cell exhaustion even in the context of a Th2 cytokine profile [52].

Th17 AND T_{regs}

Th17 and T_{regs} are T cell subsets that share many of the same differentiation pathways, despite having very different effects on adaptive immunity. Whereas T_{regs} promote an anti-inflammatory response, Th17 cells promote a proinflammatory response that has been implicated in autoimmunity. The role of Th17 cells in response to microbial infections is thought to clear pathogens that are uncontrolled by Th1 and Th2. The importance of Th17 cells was first elucidated in infectious diseases, such as tuberculosis [53] and leishmaniasis [54], and more recently is demonstrating a significant role in cancer pathogenesis [55]. Th17 cells uniquely secrete IL-17, and this T cell subset is associated with the transcription factor RORc [56]. It is thought that the imbalance between $Foxp3^+$ T_{regs} and Th17 cells plays a role in any immune-related disease. On 1 hand, Th17 is a $CD4^+$ T cell subset that plays a role in protecting the host by secreting proinflammatory cytokines. On the other hand, $Foxp3^+$ T_{regs} play a role in suppressing immune response. The roles of these T cell subsets were made more interesting by the discovery T cells expressing IL-17 and Foxp3. One thought is that T_{regs} can be therapeutically targeted with a complex mixture of inflammatory cytokines to promote the expression of IL-17 and hence, diminish the immunosuppressive effects of T_{regs} [57].

IL-17 has been well characterized in the context of tuberculosis. Th17 differentiation is primarily driven by IL-23 and plays a role in the early control of *M. tuberculosis* infection through granuloma formation and proper neutrophil recruitment to the site of infection [58, 59]. IL-17 also has a role in the early infection of leprosy. Healthy household contacts with a long-term exposure to patients with leprosy showed the highest expression of IL-17 compared with

unexposed individuals, suggesting that IL-17 plays a role in the prevention of disease. Patients with TL PBMC had higher IL-17, IL-21, IL-22, IL-23, and the associated RORc transcription factor expression compared with patients with LL [60].

Whereas STAT3 expression, an essential factor in the differentiation of Th17 cells, remains similar between patients with LL and TL, phosphorylated STAT3 is found at higher levels in patients with TL compared with patients with LL [60]. Simply put, severe forms of leprosy are associated with defective levels of IL-17 and an overproduction of the factors that lead to the differentiation of T_{regs} [61]. The distinct role of IL-17 in cancer, however, seems less certain, as recent studies have demonstrated the plasticity of this T cell subset with heterogeneous functions that may be tissue specific [62].

T_{reg}-mediated immune response is 1 reason why anti-tumor treatments have been cited to fail, promoting tumor cells to evade immune surveillance. T_{regs} are a subset of T cells that are involved in suppressing immune response, typically to inhibit excessive immune reactions. They express CD4, CD25, and Foxp3, which can be genetically defective in autoimmune and inflammatory diseases [63]. The role of Foxp3 in tumor cells remains controversial, but it is thought that its overexpression is linked to metastatic spread in some cancers [64]. A subset of T_{regs}, iT_{reg}, is thought to mediate suppression on effector T cells, notably through the secretion of TGF-β and PGE_2, providing tumor resistance to apoptosis or antitumor treatments [65]. The T_{regs} that are derived from specific tumors are known to have comparatively higher suppressive activity [66, 67].

Foxp3 expression in T_{regs} has also been link to the *M. leprae* proliferation seen in patients with LL, who are found to have higher frequencies of $CD25^+$ T_{reg}, TGF-β, and Foxp3 expression linked with anti-inflammatory macrophages [68, 69]. Skin lesions and antigen-stimulated PBMC showed increased gene expression of TGF-β in LL compared with TL [69]. $Foxp3^+$ iT_{regs} may work alongside TGF-β to down-regulate T cell responses, leading to the antigen-specific anergy associated with LL. TGF-β produced in tumor cells also aids in the conversion of the TL-predominant $CD4^+$ T cell to suppressive T_{regs} [70]. Patients with BT leprosy and patients with LL have increased interaction of Foxp3 with histone deacetylase 7/9 in the nuclei of $CD4^+CD25^+$ T cells, leading to immune suppression and progression of disease. This interaction appears to modulate CTLA-4 expression, as Foxp3 mRNA silencing in PBMCs from these patients down-regulated CTLA-4 gene expression and as Foxp3 directly binds to the promoter regions of CTLA-4 and histone acetyl transferase [71].

NEGATIVE CHECKPOINT INHIBITORS AND ENL

Patients with leprosy commonly develop a reaction known as type 2 ENL after the initiation of multidrug therapy. Approximately 1 in 4 of these patients will present with a type 2 ENL that masquerades as an autoimmune disease, such as rheumatoid arthritis, lupus erythematosus, or antiphospholipid syndrome [72]. The current standard of treatment for type 2 ENL is thalidomide, an immunomodulatory agent that has a dual property of being both anti-inflammatory whereas concomitantly enhancing Th1 cell immunity.

The advent of ipilimumab and other checkpoint inhibitors has changed the field of oncology and made immunotherapy of stage IV cancer a consideration for first-line therapy. The side effects of ipilimumab and other checkpoint inhibitors are similar to the ENL type 2 reaction of leprosy and include a variety of autoimmune syndromes, including inflammatory bowel disease, pneumonitis, hypophysitis, and thyroiditis [73].

Thalidomide and other immunomodulatory drugs are approved by the FDA for multiple myeloma and are often used off label for other cancers. Therefore, the combination of thalidomide and checkpoint inhibitors should be considered in cancer immunotherapy, as the acute inflammatory reactions against neoplastic cells are desirable for tumor destruction but require control of the autoimmune side effects.

NCR: CTLA-4

CTLA-4 is a CD28 homolog that is transiently expressed on activated T cells. CTLA-4 binds to B7-1 (CD80) and B7-2 (CD86) to deliver a negative immunoregulatory signal that leads to T cell anergy. Mice with a deficiency in the CTLA-4 gene develop a rampant cell-mediated immune response, leading to a fatal systemic lymphoproliferative disease with multiorgan lymphocytic infiltration and damage [74]. Typically, early T cell activation following binding of CD28 leads to an up-regulation in the expression of CTLA-4, limiting the extent of T cell activation by competition with CD28. An excess of CTLA-4 signaling may be a cause for the unresponsiveness of T cells seen with LL, with clones that expressed $CD8^+CD28^-CTLA\text{-}4^+$ T cells [75].

Ipilimumab was developed as the first anti-CTLA-4 mAb that was FDA-approved for the treatment of metastatic melanoma [76]. Although it is thought that the mechanism of action of ipilimumab is through inactivating CTLA-4 and thereby liberating an anti-tumor T cell-mediated response, there are some who believe that the drug works by inhibiting T_{regs} in the tumor microenvironment [3].

CTLA-4 is present at much higher levels in cells from patients with LL compared with patients with TL [75]. The addition of anti-CTLA-4-blocking antibodies to PBMCs from patients with TL had little effect on patients with TL but had a stimulatory or inhibitory effect on the proliferation of PBMC from patients with LL [75]. The effects of CTLA-4 in suppressing T cell activation may be mediated by Foxp3, TGF-β, and Cbl-b [77]. Increased interaction of Foxp3 in $CD4^+CD25^+$ T cells from patients with BT and LL is thought to be responsible for the Foxp3-driven immune suppression during LL-like conditions. In these patients, the binding of Foxp3 to the promoter of CTLA-4 genes led to the downstream effects of Foxp3 [71]. Another downstream event from CTLA-4 that produces T cell anergy is thought to be Cbl-b. TGF-β and CTLA-4 increase the prevalence of LL progression through Cbl-b [14].

It is poorly understood how the interaction between CTLA-4 and ligand CD86 produces lepromatous-like conditions through the induction of T_{regs}. Patients with LL are deficient in CD86 and yet still maintain an increase in T_{regs}, both in situ and in vitro [78], and an increase in IL-10; this is the opposite of what it seen in TL [78].

An experiment done in cells from patients with TL shows that the costimulatory CD86, but not CD80, plays a crucial role in the

presentation of the leprosy antigen in monocytes, as demonstrated through the use of neutralizing antibodies [15]. CD86 was also found to be highly expressed in healthy individuals exposed to *M. leprae* compared with patients with TL and is thought to be protective against disease. These patients were also found to have higher levels of CD28, the receptor for the CD86-driven costimulation [75]. In contrast, patients with LL had a deficiency in CD86. It is noteworthy that the activation of T cell clones from tuberculoid lesions was not blocked by anti-CD28 or anti-CTLA-4 antibodies but was blocked by anti-B7-1 antibodies, suggesting that B7-1 may use another costimulatory pathway. In contrast, LL lesions were strongly regulated by CD28 during T cell activation [75].

NCR: PD-1

PD-1 is another essential NCR that reduces cell-mediated immunity. Currently, an anti-PD-1 mAb, nivolumab, is being used to prolong the survival rate of patients with metastatic melanoma, often in combination with immunosensitizing agents [79]. Normally, PD-1 is up-regulated by T and B cells within 24 h following their activation [80]. Following the formation of PD-1, PD-1 forms negative costimulatory microclusters associated with TCRs and Src homology 2 domain-containing tyrosine phosphatase 2, leading to T cell inhibition [81].

The ligand PD-L1 can be stimulated by IFN on T cells, macrophages, and tumors and is important in the prevention of autoimmunity but is also thought to inhibit antitumor T cell activity. High expression of PD-L1 appears associated with poorer outcomes [82]. There is evidence in the context of leprosy that the expression of PD-1 and IFN-γ leads to more favorable clinical outcomes. In a study looking at T cells from patients with TL, there was an increased display of PD-1, which is thought to modulate an overactive immune response to the pathogen [15]. Furthermore, the secretion of IFN-γ in the context of leprosy leads to better clinical outcomes in *M. leprae* infection, as IFN-γ activates downstream pathways for antimicrobial gene expression [48]. IFN-γ is preferentially expressed in TL. In contrast, type 2 IFN, IFN-β, and its downstream genes, including IL-10, were induced in monocytes by *M. leprae* in vitro and preferentially expressed in LL [48].

NCR: VISTA

VISTA (also known as PD-1 homolog) was recently discovered as an NCR that suppresses T cell activation. Structurally, VISTA is most similar to PD-1. There are functional differences in VISTA and PD-1; however, whereas knockout-mice for both NCR led to chronic inflammation and spontaneous activation of T cells in mice, the double knockout led to significantly worse phenotypes [83].

VISTA is expressed more on hematopoietic cells with a weaker expression on T cells. Yet, T cells still express and respond to VISTA and lead to the suppression of T cell activation, proliferation, and cytokine production [84, 85]. A loss-of-function mutation in VISTA lowers the threshold for T cell activation—an important discovery in understanding autoimmune disease and a potential strategy for future cancer immunotherapies. VISTA antibodies have been suggested as adjuvant therapy in combination with other negative checkpoint inhibitors, as it appears to have unique, nonredundant, downstream effects [83].

It is noteworthy that both naïve and antigen-experienced T cells are suppressed by the presence of VISTA, suggesting that the receptor for VISTA is constitutively expressed, even in inactivated T cells [84]. Because of this, it is thought that VISTA prevents the overactivation of T cells in response to self-antigens. Mice lacking VISTA expression have greater levels of spontaneous T cell activation and a phenotype with greater inflammatory cytokine and chemokine expression [86]. VISTA knockout mice showed higher frequency of activated peripheral T cells, with increased populations of CD44 high CD62 ligand low T cells, as well as the production of IFN-γ, TNF-α, and IL-17A. These data show a Th1-polarizing response in VISTA knockout mice compared with controls [85]. One study showed that VISTA was highly expressed in myeloid cells and $Foxp3^{+}CD4^{+}$ T_{regs} but not in the tumor microenvironment. Nonetheless, a VISTA blockage still altered the immune inhibition seen in the tumor microenvironment by increasing the presence of activated dendritic cells and reducing tumor-specific T_{regs}. A VISTA mAb was successfully used to suppress the growth of melanoma in mouse models [87].

There is potential for knowledge to be furthered by studying VISTA in the context of infectious diseases. VISTA is modulated by some TLRs, most notably up-regulated by TLR3 and TLR5 and down-regulated by TLR8/9. The TLR associated with an innate immune response to leprosy, TLR2/1, was found to have no effect on the expression of VISTA [88]. Research in HIV has shown that the overexpression of VISTA has led to spontaneous secretions of an array of cytokines but also the depletion of $CD4^{+}$ T cells, possibly associated with the pathogenesis of the disease or the modulatory roles of VISTA. Despite TLR2/1 likely not being involved in VISTA expression, the leprosy spectrum still provides a model for understanding the role of VISTA in T cell regulation. The deletion or blockage of NCR as VISTA leads to a Th1 polarization that resembles a TL patient profile. In the context of TL, Th1 responses activate macrophages and inhibit the growth of pathogens, resulting in a self-curing disease [75]. VISTA is a new NCR that has only come to the scene within the past half-decade. Future research into the properties of this NCR, using the leprosy model, can provide greater insight into the future potential of this target, as well as its role in cancer and autoimmunity.

CONCLUSION

The development of negative checkpoint inhibitors, such as ipilimumab and nivolumab, has brought immunotherapy of stage IV advanced-staged cancers to potential first-line therapy. In short, these treatment options allow for a more-effective immune response against tumors that have previously managed to escape a T cell immune response. The mechanism by which these effective medications work, however, remains elusive. As leprosy is a disease that presents as a spectrum, not only of clinical manifestations but also of T cell response, this disease is an ideal model to understand further the mechanisms that underlie cancer immunotherapy and generally, immunosurveillance. Similar to a progressive and proliferating tumor, *M. leprae* escapes immunosurveillance in more-severe forms of leprosy—lepromatous and borderline LL. The mechanisms by which *M. leprae* is able to evade the host immune response involve alterations in

lipid bodies, miRNAs, and differentiation of Schwann cells and may also involve the modulation of immune regulators, such as CTLA-4, PD-1, and VISTA. The understanding of how this pathogen regulates T cell plasticity between anergy and immunosurveillance will provide valuable insight into future knowledge and development of cancer immunotherapy.

AUTHORSHIP

A.J.P. wrote the manuscript and prepared the figure. T.R., F.M., and W.R.L. edited and contributed to the content of the manuscript. We thank Dr. Randolph J. Noelle (Geisel School of Medicine at Dartmouth) and Dr. K. Mark Ansel (University of California, San Francisco) for their contributions to the discussion of VISTA and miRNA regulation of T cell subsets. This work was supported by the U.S. Public Health Service and the National Hansen's Disease Program.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

1. Couzin-Frankel, J. (2013) Breakthrough of the year 2013. Cancer immunotherapy. *Science* **342,** 1432–1433.
2. Burnet, F. M. (1970) The concept of immunological surveillance. *Prog. Exp. Tumor Res.* **13,** 1–27.
3. Lines, J. L., Sempere, L. F., Broughton, T., Wang, L., Noelle, R. (2014) VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy. *Cancer Immunol. Res.* **2,** 510–517.
4. June, C. H., Ledbetter, J. A., Gillespie, M. M., Lindsten, T., Thompson, C. B. (1987) T-Cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. *Mol. Cell. Biol.* **7,** 4472–4481.
5. Trinchieri, G. (2015) Cancer immunity: lessons from infectious diseases. *J. Infect. Dis.* **212** (Suppl 1), S67–S73.
6. Chandler, D. J., Hansen, K. S., Mahato, B., Darlong, J., John, A., Lockwood, D. N. (2015) Household costs of leprosy reactions (ENL) in rural India. *PLoS Negl. Trop. Dis.* **9,** e0003431.
7. Bloom, B. R. (1986) Learning from leprosy: a perspective on immunology and the Third World. *J. Immunol.* **137,** i–x.
8. Modlin, R. L., Melancon-Kaplan, J., Young, S. M., Pirmez, C., Kino, H., Convit, J., Rea, T. H., Bloom, B. R. (1988) Learning from lesions: patterns of tissue inflammation in leprosy. *Proc. Natl. Acad. Sci. USA* **85,** 1213–1217.
9. Fink, M. P. (2010) Editorial: Lepromatous leprosy, lipids, and lessons in immunology: what we can learn by using modern methods to study an ancient disease. *J. Leukoc. Biol.* **87,** 361–363.
10. Ridley, D. S. (1974) Histological classification and the immunological spectrum of leprosy. *Bull. World Health Organ.* **51,** 451–465.
11. Collins, A. V., Brodie, D. W., Gilbert, R. J., Iaboni, A., Manso-Sancho, R., Walse, B., Stuart, D. I., van der Merwe, P. A., Davis, S. J. (2002) The interaction properties of costimulatory molecules revisited. *Immunity* **17,** 201–210.
12. Modlin, R. L. (2010) The innate immune response in leprosy. *Curr. Opin. Immunol.* **22,** 48–54.
13. Abbas, A. K. (2011) A network of regulatory pathways in lepromatous leprosy. *Clin. Immunol.* **141,** 127.
14. Kumar, S., Naqvi, R. A., Khanna, N., Pathak, P., Rao, D. N. (2011) Th3 immune responses in the progression of leprosy via molecular cross-talks of TGF-β, CTLA-4 and Cbl-b. *Clin. Immunol.* **141,** 133–142.
15. Palermo, Mde. L., Trindade, M. A., Duarte, A. J., Cacere, C. R., Benard, G. (2012) Differential expression of the costimulatory molecules CD86, CD28, CD152 and PD-1 correlates with the host-parasite outcome in leprosy. *Mem. Inst. Oswaldo Cruz* **107** (Suppl 1), 167–173.
16. Modlin, R. L. (2002) Learning from leprosy: insights into contemporary immunology from an ancient disease. *Skin Pharmacol. Appl. Skin Physiol.* **15,** 1–6.
17. Mittal, D., Gubin, M. M., Schreiber, R. D., Smyth, M. J. (2014) New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. *Curr. Opin. Immunol.* **27,** 16–25.
18. Dunn, G. P., Old, L. J., Schreiber, R. D. (2004) The three Es of cancer immunoediting. *Annu. Rev. Immunol.* **22,** 329–360.
19. Kim, R., Emi, M., Tanabe, K. (2007) Cancer immunoediting from immune surveillance to immune escape. *Immunology* **121,** 1–14.
20. Leisegang, M., Kammertoens, T., Uckert, W., Blankenstein, T. (2016) Targeting human melanoma neoantigens by T cell receptor gene therapy. *J. Clin. Invest.* **126,** 854–858.
21. Burgess, D. J. (2012) Tumour immunogenicity: editorial selection demystified. *Nat. Rev. Cancer* **12,** 227.
22. Matsushita, H., Vesely, M. D., Koboldt, D. C., Rickert, C. G., Uppaluri, R., Magrini, V. J., Arthur, C. D., White, J. M., Chen, Y. S., Shea, L. K., Hundal, J., Wendl, M. C., Demeter, R., Wylie, T., Allison, J. P., Smyth, M. J., Old, L. J., Mardis, E. R., Schreiber, R. D. (2012) Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature* **482,** 400–404.
23. Tomasi, T. B., Magner, W. J., Khan, A. N. (2006) Epigenetic regulation of immune escape genes in cancer. *Cancer Immunol. Immunother.* **55,** 1159–1184.
24. Mattos, K. A., D'Avila, H., Rodrigues, L. S., Oliveira, V. G., Sarno, E. N., Atella, G. C., Pereira, G. M., Bozza, P. T., Pessolani, M. C. (2010) Lipid droplet formation in leprosy: Toll-like receptor-regulated organelles involved in eicosanoid formation and *Mycobacterium leprae* pathogenesis. *J. Leukoc. Biol.* **87,** 371–384.
25. Marques, M. A., Neves-Ferreira, A. G., da Silveira, E. K., Valente, R. H., Chapeaurouge, A., Perales, J., da Silva Bernardes, R., Dobos, K. M., Spencer, J. S., Brennan, P. J., Pessolani, M. C. (2008) Deciphering the proteomic profile of *Mycobacterium leprae* cell envelope. *Proteomics* **8,** 2477–2491.
26. Schrader, M., Fahimi, H. D. (2006) Peroxisomes and oxidative stress. *Biochim. Biophys. Acta* **1763,** 1755–1766.
27. Cha, M. K., Suh, K. H., Kim, I. H. (2009) Overexpression of peroxiredoxin I and thioredoxin1 in human breast carcinoma. *J. Exp. Clin. Cancer Res.* **28,** 93.
28. Lepore, M., de Lalla, C., Gundimeda, S. R., Gsellinger, H., Consonni, M., Garavaglia, C., Sansano, S., Piccolo, F., Scelfo, A., Häussinger, D., Montagna, D., Locatelli, F., Bonini, C., Bondanza, A., Forcina, A., Li, Z., Ni, G., Ciceri, F., Jenö, P., Xia, C., Mori, L., Dellabona, P., Casorati, G., De Libero, G. (2014) A novel self-lipid antigen targets human T cells against CD1c(+) leukemias. *J. Exp. Med.* **211,** 1363–1377.
29. Sieling, P. A., Jullien, D., Dahlem, M., Tedder, T. F., Rea, T. H., Modlin, R. L., Porcelli, S. A. (1999) CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. *J. Immunol.* **162,** 1851–1858.
30. Masaki, T., Qu, J., Cholewa-Waclaw, J., Burr, K., Raaum, R., Rambukkana, A. (2013) Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection. *Cell* **152,** 51–67.
31. Binder, Z. A., Siu, I. M., Eberhart, C. G., Ap Rhys, C., Bai, R. Y., Staedtke, V., Zhang, H., Smoll, N. R., Piantadosi, S., Piccirillo, S. G., Dimeco, F., Weingart, J. D., Vescovi, A., Olivi, A., Riggins, G. J., Gallia, G. L. (2013) Podocalyxin-like protein is expressed in glioblastoma multiforme stem-like cells and is associated with poor outcome. *PLoS One* **8,** e75945.
32. Harapan, H., Fitra, F., Ichsan, I., Mulyadi, M., Miotto, P., Hasan, N. A., Calado, M., Cirillo, D. M. (2013) The roles of microRNAs on tuberculosis infection: meaning or myth? *Tuberculosis (Edinb.)* **93,** 596–605.
33. Liang, Y., Pan, H. F., Ye, D. Q. (2015) microRNAs function in $CD8^+$ T cell biology. *J. Leukoc. Biol.* **97,** 487–497.
34. Cezar-de-Mello, P. F., Toledo-Pinto, T. G., Marques, C. S., Arnez, L. E., Cardoso, C. C., Guerreiro, L. T., Antunes, S. L., Jardim, M. M., Covas, Cde. J., Illaramendi, X., Dias-Baptista, I. M., Rosa, P. S., Durães, S. M., Pacheco, A. G., Ribeiro-Alves, M., Sarno, E. N., Moraes, M. O. (2014) Pre-miR-146a (rs2910164 G>C) single nucleotide polymorphism is genetically and functionally associated with leprosy. *PLoS Negl. Trop. Dis.* **8,** e3099.
35. Liu, P. T., Wheelwright, M., Teles, R., Komisopoulou, E., Edfeldt, K., Ferguson, B., Mehta, M. D., Vazirnia, A., Rea, T. H., Sarno, E. N., Graeber, T. G., Modlin, R. L. (2012) MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. *Nat. Med.* **18,** 267–273.
36. Sheedy, F. J. (2015) Turning 21: induction of miR-21 as a key switch in the inflammatory response. *Front. Immunol.* **6,** 19.
37. Yang, X., Zeng, Z., Hou, Y., Yuan, T., Gao, C., Jia, W., Yi, X., Liu, M. (2014) MicroRNA-92a as a potential biomarker in diagnosis of colorectal cancer: a systematic review and meta-analysis. *PLoS One* **9,** e88745.
38. Schwartz, R. H. (1990) A cell culture model for T lymphocyte clonal anergy. *Science* **248,** 1349–1356.
39. Behar, S. M., Carpenter, S. M., Booty, M. G., Barber, D. L., Jayaraman, P. (2014) Orchestration of pulmonary T cell immunity during *Mycobacterium tuberculosis* infection: immunity interruptus. *Semin. Immunol.* **26,** 559–577.
40. Balkhi, M. Y., Ma, Q., Ahmad, S., Junghans, R. P. (2015) T Cell exhaustion and interleukin 2 downregulation. *Cytokine* **71,** 339–347.
41. Riley, J. L. (2009) PD-1 signaling in primary T cells. *Immunol. Rev.* **229,** 114–125.
42. Wu, K., Kryczek, I., Chen, L., Zou, W., Welling, T. H. (2009) Kupffer cell suppression of $CD8^+$ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res.* **69,** 8067–8075.
43. Curiel, T. J., Wei, S., Dong, H., Alvarez, X., Cheng, P., Mottram, P., Krzysiek, R., Knutson, K. L., Daniel, B., Zimmermann, M. C., David, O., Burow, M., Gordon, A., Dhurandhar, N., Myers, L., Berggren, R., Hemminki, A., Alvarez, R. D., Emilie, D., Curiel, D. T., Chen, L., Zou, W. (2003) Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat. Med.* **9,** 562–567.
44. Baitsch, L., Baumgaertner, P., Devêvre, E., Raghav, S. K., Legat, A., Barba, L., Wieckowski, S., Bouzourene, H., Deplancke, B., Romero, P., Rufer, N.,

Speiser, D. E. (2011) Exhaustion of tumor-specific CD8$^+$ T cells in metastases from melanoma patients. *J. Clin. Invest.* **121,** 2350–2360.
45. Meloni, F., Morosini, M., Solari, N., Passadore, I., Nascimbene, C., Novo, M., Ferrari, M., Cosentino, M., Marino, F., Pozzi, E., Fietta, A. M. (2006) Foxp3 expressing CD4$^+$ CD25$^+$ and CD8$^+$CD28− T regulatory cells in the peripheral blood of patients with lung cancer and pleural mesothelioma. *Hum. Immunol.* **67,** 1–12.
46. Tsukishiro, T., Donnenberg, A. D., Whiteside, T. L. (2003) Rapid turnover of the CD8(+)CD28(−) T-cell subset of effector cells in the circulation of patients with head and neck cancer. *Cancer Immunol. Immunother.* **52,** 599–607.
47. Pedroza-Gonzalez, A., Xu, K., Wu, T. C., Aspord, C., Tindle, S., Marches, F., Gallegos, M., Burton, E. C., Savino, D., Hori, T., Tanaka, Y., Zurawski, S., Zurawski, G., Bover, L., Liu, Y. J., Banchereau, J., Palucka, A. K. (2011) Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. *J. Exp. Med.* **208,** 479–490.
48. Teles, R. M., Graeber, T. G., Krutzik, S. R., Montoya, D., Schenk, M., Lee, D. J., Komisopoulou, E., Kelly-Scumpia, K., Chun, R., Iyer, S. S., Sarno, E. N., Rea, T. H., Hewison, M., Adams, J. S., Popper, S. J., Relman, D. A., Stenger, S., Bloom, B. R., Cheng, G., Modlin, R. L. (2013) Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science* **339,** 1448–1453.
49. Renauld, J. C., van der Lugt, N., Vink, A., van Roon, M., Godfraind, C., Warnier, G., Merz, H., Feller, A., Berns, A., Van Snick, J. (1994) Thymic lymphomas in interleukin 9 transgenic mice. *Oncogene* **9,** 1327–1332.
50. Purwar, R., Schlapbach, C., Xiao, S., Kang, H. S., Elyaman, W., Jiang, X., Jetten, A. M., Khoury, S. J., Fuhlbrigge, R. C., Kuchroo, V. K., Clark, R. A., Kupper, T. S. (2012) Robust tumor immunity to melanoma mediated by interleukin-9-producing T cells. *Nat. Med.* **18,** 1248–1253.
51. Wu, B., Huang, C., Kato-Maeda, M., Hopewell, P. C., Daley, C. L., Krensky, A. M., Clayberger, C. (2008) IL-9 is associated with an impaired Th1 immune response in patients with tuberculosis. *Clin. Immunol.* **126,** 202–210.
52. Finiasz, M. R., Franco, M. C., de la Barrera, S., Rutitzky, L., Pizzariello, G., del Carmen Sasiain, M., Renauld, J. C., Van Snick, J., Fink, S. (2007) IL-9 promotes anti-*Mycobacterium leprae* cytotoxicity: involvement of IFN-γ. *Clin. Exp. Immunol.* **147,** 139–147.
53. Umemura, M., Yahagi, A., Hamada, S., Begum, M. D., Watanabe, H., Kawakami, K., Suda, T., Sudo, K., Nakae, S., Iwakura, Y., Matsuzaki, G. (2007) IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis bacille* Calmette-Guerin infection. *J. Immunol.* **178,** 3786–3796.
54. Gonzalez-Lombana, C., Gimblet, C., Bacellar, O., Oliveira, W. W., Passos, S., Carvalho, L. P., Goldschmidt, M., Carvalho, E. M., Scott, P. (2013) IL-17 mediates immunopathology in the absence of IL-10 following *Leishmania major* infection. *PLoS Pathog.* **9,** e1003243.
55. Kryczek, I., Banerjee, M., Cheng, P., Vatan, L., Szeliga, W., Wei, S., Huang, E., Finlayson, E., Simeone, D., Welling, T. H., Chang, A., Coukos, G., Liu, R., Zou, W. (2009) Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. *Blood* **114,** 1141–1149.
56. Unutmaz, D. (2009) RORC2: the master of human Th17 cell programming. *Eur. J. Immunol.* **39,** 1452–1455.
57. Du, R., Zhao, H., Yan, F., Li, H. (2014) IL-17$^+$Foxp3$^+$ T cells: an intermediate differentiation stage between Th17 cells and regulatory T cells. *J. Leukoc. Biol.* **96,** 39–48.
58. Gaffen, S. L., Jain, R., Garg, A. V., Cua, D. J. (2014) The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat. Rev. Immunol.* **14,** 585–600.
59. Cruz, A., Khader, S. A., Torrado, E., Fraga, A., Pearl, J. E., Pedrosa, J., Cooper, A. M., Castro, A. G. (2006) Cutting edge: IFN-γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. *J. Immunol.* **177,** 1416–1420.
60. Saini, C., Ramesh, V., Nath, I. (2013) CD4$^+$ Th17 cells discriminate clinical types and constitute a third subset of non–Th1, non–Th2 T cells in human leprosy. *PLoS Negl. Trop. Dis.* **7,** e2338.
61. Attia, E. A., Abdallah, M., El-Khateeb, E., Saad, A. A., Lotfi, R. A., Abdallah, M., El-Shennawy, D. (2014) Serum Th17 cytokines in leprosy: correlation with circulating CD4(+) CD25 (high)FoxP3 (+) T-regs cells, as well as down regulatory cytokines. *Arch. Dermatol. Res.* **306,** 793–801.
62. Sundrud, M. S., Trivigno, C. (2013) Identity crisis of Th17 cells: many forms, many functions, many questions. *Semin. Immunol.* **25,** 263–272.
63. Hori, S., Nomura, T., Sakaguchi, S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299,** 1057–1061.
64. Triulzi, T., Tagliabue, E., Balsari, A., Casalini, P. (2013) FOXP3 expression in tumor cells and implications for cancer progression. *J. Cell. Physiol.* **228,** 30–35.
65. Whiteside, T. L. (2012) What are regulatory T cells (Treg) regulating in cancer and why? *Semin. Cancer Biol.* **22,** 327–334.
66. Yokokawa, J., Cereda, V., Remondo, C., Gulley, J. L., Arlen, P. M., Schlom, J., Tsang, K. Y. (2008) Enhanced functionality of CD4$^+$CD25 (high)FoxP3$^+$ regulatory T cells in the peripheral blood of patients with prostate cancer. *Clin. Cancer Res.* **14,** 1032–1040.
67. Gasparoto, T. H., de Souza Malaspina, T. S., Benevides, L., de Melo, Jr., E. J., Costa, M. R., Damante, J. H., Ikoma, M. R., Garlet, G. P., Cavassani, K. A., da Silva, J. S., Campanelli, A. P. (2010) Patients with oral squamous cell carcinoma are characterized by increased frequency of suppressive regulatory T cells in the blood and tumor microenvironment. *Cancer Immunol. Immunother.* **59,** 819–828.
68. Bobosha, K., Wilson, L., van Meijgaarden, K. E., Bekele, Y., Zewdie, M., van der Ploeg-van Schip, J. J., Abebe, M., Hussein, J., Khadge, S., Neupane, K. D., Hagge, D. A., Jordanova, E. S., Aseffa, A., Ottenhoff, T. H., Geluk, A. (2014) T-Cell regulation in lepromatous leprosy. *PLoS Negl. Trop. Dis.* **8,** e2773.
69. Saini, C., Ramesh, V., Nath, I. (2014) Increase in TGF-β secreting CD4$^+$CD25$^+$ FOXP3$^+$ T regulatory cells in anergic lepromatous leprosy patients. *PLoS Negl. Trop. Dis.* **8,** e2639.
70. Nishikawa, H., Sakaguchi, S. (2010) Regulatory T cells in tumor immunity. *Int. J. Cancer* **127,** 759–767.
71. Kumar, S., Naqvi, R. A., Ali, R., Rani, R., Khanna, N., Rao, D. N. (2014) FoxP3 provides competitive fitness to CD4$^+$CD25$^+$ T cells in leprosy patients via transcriptional regulation. *Eur. J. Immunol.* **44,** 431–439.
72. Martiniuk, F., Giovinazzo, J., Tan, A. U., Shahidullah, R., Haslett, P., Kaplan, G., Levis, W. R. (2012) Lessons of leprosy: the emergence of TH17 cytokines during type II reactions (ENL) is teaching us about T-cell plasticity. *J. Drugs Dermatol.* **11,** 626–630.
73. Cranmer, L. D., Hersh, E. (2007) The role of the CTLA4 blockade in the treatment of malignant melanoma. *Cancer Invest.* **25,** 613–631.
74. Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., Nomura, T., Sakaguchi, S. (2008) CTLA-4 control over Foxp3$^+$ regulatory T cell function. *Science* **322,** 271–275.
75. Schlienger, K., Uyemura, K., Jullien, D., Sieling, P. A., Rea, T. H., Linsley, P. S., Modlin, R. L. (1998) B7-1, but not CD28, is crucial for the maintenance of the CD4$^+$ T cell responses in human leprosy. *J. Immunol.* **161,** 2407–2413.
76. Cameron, F., Whiteside, G., Perry, C. (2011) Ipilimumab: first global approval. *Drugs* **71,** 1093–1104.
77. Wallner, S., Gruber, T., Baier, G., Wolf, D. (2012) Releasing the brake: targeting Cbl-b to enhance lymphocyte effector functions. *Clin. Dev. Immunol.* **2012,** 692639.
78. Palermo, M. L., Pagliari, C., Trindade, M. A., Yamashitafuji, T. M., Duarte, A. J., Cacere, C. R., Benard, G. (2012) Increased expression of regulatory T cells and down-regulatory molecules in lepromatous leprosy. *Am. J. Trop. Med. Hyg.* **86,** 878–883.
79. Fujimura, T., Furudate, S., Kakizaki, A., Kambayashi, Y., Haga, T., Hashimoto, A., Aiba, S. (2015) Contact immunotherapy enhances the therapeutic effects of nivolumab in treating in-transit melanoma: two cases reports. *J. Dermatol.* **42,** 1–4.
80. Agata, Y., Kawasaki, A., Nishimura, H., Ishida, Y., Tsubata, T., Yagita, H., Honjo, T. (1996) Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int. Immunol.* **8,** 765–772.
81. Yokosuka, T., Takamatsu, M., Kobayashi-Imanishi, W., Hashimoto-Tane, A., Azuma, M., Saito, T. (2012) Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J. Exp. Med.* **209,** 1201–1217.
82. Flies, D. B., Chen, L. (2006) Modulation of immune response by B7 family molecules in tumor microenvironments. *Immunol. Invest.* **35,** 395–418.
83. Liu, J., Yuan, Y., Chen, W., Putra, J., Suriawinata, A. A., Schenk, A. D., Miller, H. E., Guleria, I., Barth, R. J., Huang, Y. H., Wang, L. (2015) Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses. *Proc. Natl. Acad. Sci. USA* **112,** 6682–6687.
84. Wang, L., Rubinstein, R., Lines, J. L., Wasiuk, A., Ahonen, C., Guo, Y., Lu, L. F., Gondek, D., Wang, Y., Fava, R. A., Fiser, A., Almo, S., Noelle, R. J. (2011) VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. *J. Exp. Med.* **208,** 577–592.
85. Wang, L., Le Mercier, I., Putra, J., Chen, W., Liu, J., Schenk, A. D., Nowak, E. C., Suriawinata, A. A., Li, J., Noelle, R. J. (2014) Disruption of the immune-checkpoint VISTA gene imparts a proinflammatory phenotype with predisposition to the development of autoimmunity. *Proc. Natl. Acad. Sci. USA* **111,** 14846–14851.
86. Flies, D. B., Han, X., Higuchi, T., Zheng, L., Sun, J., Ye, J. J., Chen, L. (2014) Coinhibitory receptor PD-1H preferentially suppresses CD4$^+$ T cell-mediated immunity. *J. Clin. Invest.* **124,** 1966–1975.
87. Le Mercier, I., Chen, W., Lines, J. L., Day, M., Li, J., Sergent, P., Noelle, R. J., Wang, L. (2014) VISTA regulates the development of protective antitumor immunity. *Cancer Res.* **74,** 1933–1944.
88. Bharaj, P., Chahar, H. S., Alozie, O. K., Rodarte, L., Bansal, A., Goepfert, P. A., Dwivedi, A., Manjunath, N., Shankar, P. (2014) Characterization of programmed death-1 homologue-1 (PD-1H) expression and function in normal and HIV infected individuals. *PLoS One* **9,** e109103.
89. Ridley, D. S., Jopling, W. H. (1962) A classification of leprosy for research purposes. *Lepr. Rev.* **33,** 119–128.
90. Koebel, C. M., Vermi, W., Swann, J. B., Zerafa, N., Rodig, S. J., Old, L. J., Smyth, M. J., Schreiber, R. D. (2007) Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* **450,** 903–907.

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