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REVIEW

# Immune responsiveness and protective immunity after transplantation

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## Summary

The growing success of solid organ transplantation poses unique challenges for the implementation of effective immunization strategies. Although live attenuated vaccines have proven benefits for the general population, immunosuppressed patients are at risk for unique complications such as infection from the vaccine because of lack of both clearance and containment of a live attenuated virus. Moreover, while vaccination strategies using killed organisms or purified peptides are believed to be safe for immunosuppressed patients, they may have reduced efficacy in this population. The current lack of knowledge of the basic safety and efficacy of vaccination strategies in the immunosuppressed has limited the development of guidelines regarding vaccination in this population. Recent fears of influenza pandemics and potential attacks by weaponized pathogens such as smallpox heighten the need for increased knowledge. Herein, we review the current understanding of the effects of immunosuppressants on the immune system and the ability of the suppressed immune system to respond to vaccination. This review highlights the need for systematic, longitudinal studies in both humans and nonhuman primates to understand better the defects in innate and adaptive immunity in transplant recipients, thereby aiding the development of strategies to vaccinate these individuals.

## Introduction

For selected patients with organ failure, transplantation is the preferred treatment modality. Over 25 000 solid organ transplants are performed each year in the United States, and 15 000 of these are renal transplants. The number of transplant recipients in the United States has more than doubled in the past decade, now exceeding 150 000 individuals [1]. This growing population of immunocompromised individuals poses unique challenge for the development of immunization and containment strategies to protect the general population from communicable pathogens such as endemic or pandemic influenza, or weaponized infectious agents as the immunosuppressive therapies that prevent rejection impair key components of the adaptive and innate immune responses. Although immunosuppression is known to attenuate the response to vaccines and preclude the use of live attenuated vaccines, few studies have described how conventional immunosuppressive regimens that may include calcineurin inhibitors (CNI), m-TOR inhibitors, anti-proliferative agents, or steroids alter the immune response of transplant recipients to a vaccine. This review summarizes the current knowledge of how conventional immunosuppressive drugs alter the immune response to vaccines. This review also provides an overview of how the mechanisms of action on the immune system by both currently used immunosuppressive agents and those in development (e.g. CD28 costimulation blockers and/or JAK3 kinase inhibitors) might alter specific immunologic responses to

 

vaccination. These issues will have clear implications for the design of vaccination strategies to protect immunosuppressed transplant recipients from outbreaks of biologic pathogens and will also affect more global biodefense strategies.

## Overview of the immunologic response to infection/vaccination

### Linking innate and adaptive immunity

To understand the response of the immune system to vaccination one must understand how the immune system identifies and contains an infection. The first line of defense against primary infection is the innate immune system, which then coordinates with the adaptive immune system, generating an effective immune response [2–4]. Natural killer (NK) and dendritic (DC) cells are important components of the innate immune system. NK cells can directly lyse infected target cells and/or secrete cytokines, such as interferon-gamma (IFN-$\gamma$), that influence the adaptive immune response while DC recognize foreign pathogens through pattern recognition receptors including Toll-like receptors (TLRs) and present antigen to the adaptive immune system [2–4].

There are multiple subsets of DCs that represent different lineages and maturation stages, and may differ in phenotype, function, and microenvironment localization [5–7]. Three major subsets of DC precursors exist in human blood: myeloid, monocytic-derived (MDDC), and plasmacytoid (pDC). Each is identifiable through distinct surface marker expression patterns and possesses distinct immunoregulatory functions. For instance, MDDCs and pDCs have been shown to induce differentially either Th1 or Th2 responses in T cells [8]. Functionally, pDC precursors are a chief source of interferon-alpha (IFN-$\alpha$) released in response to viral infections [9]. Influenced by TLR signaling, DC express distinct profiles of costimulatory molecules and cytokines that can induce pro-inflammatory or tolerogenic immune responses from cells of the adaptive immune system [10]. Potentiated by cells of the innate immune system, the antigen-specific T and B cells of the adaptive immune system differentiate into effector cells, disseminate throughout the organism, and eventually contract in number after further differentiation into memory cells.

### Immune memory

Immunological memory comprises preformed antibody, memory B cells, antibody secreting plasma cells, and memory CD4 and CD8 T cells. Pre-existing antibody directly binds to pathogens and can directly neutralize or facilitate opsonization of pathogens to prevent infection. Additional antibody production to increase protective titers is provided by memory B cells that rapidly differentiate into plasma cells upon re-activation. Memory B cells also provide an anamnestic response by replenishing the long-lived plasma cell population present in the bone marrow. The memory T-cell compartment can facilitate the elimination of pathogens from the host by rapidly proliferating, secreting inflammatory cytokines, and killing infected cells. Optimal immunity to a previously encountered pathogen requires maintenance of all memory cell types in the absence of antigen and the ability to expand and mobilize these cell types upon re-infection (Fig. 1, bold line).

### Memory B-cell maintenance

The mechanisms by which memory responses are maintained are incompletely understood. Re-exposure to antigen, because of re-infection or booster vaccination, is clearly the most effective mechanism to maintain B-cell memory. In the absence of antigenic re-exposure, however, memory B cells still can persist for decades, suggesting that long-term B-cell memory can be maintained in the absence of antigen. For example, vaccine-specific antibodies and memory B cells were detected in individuals 60 years after vaccination with the smallpox vaccine [11,12]. It was proposed that antigen trapped in immune complexes on follicular DCs was required for memory B-cell maintenance [13,14]. This theory has been challenged as memory B cells can still persist in mice, which lack the ability to form immune complexes [15]. Furthermore, alteration in the B-cell receptor to prevent recognition of cognate antigen does not prevent the long-term survival of memory B cells [16] suggesting that ongoing antigen-specific stimulation is not required for memory maintenance. However, alteration in the B-cell receptor

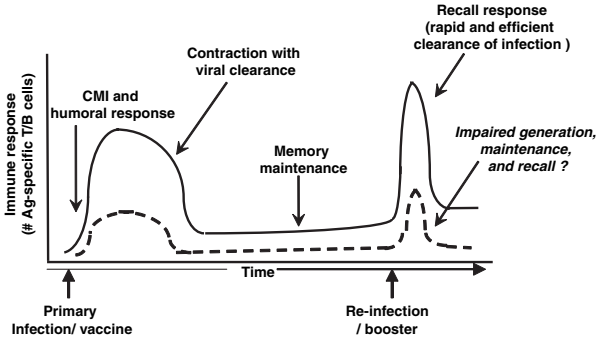


**Figure 1** Schematic representation of primary and recall immune responses to viral infection or vaccination in healthy individuals (bold line) and transplant population (dotted line). CMI, cell-mediated immunity.

so that it is nonfunctional prevents the maintenance of memory B cells. This suggests that in the absence of antigen that low-affinity, nonantigen-specific signaling through the B-cell receptor is required for maintenance of memory B cells [17]. Intermittent bystander activation, mediated by TLRs and CD4 T cell help, may be responsible for maintaining B-cell memory in humans [18].

### T-cell memory maintenance

T-cell responses following infection or vaccination occur in a series of phases (Fig. 1, bold line). In the first phase after encounter with antigen, T cells undergo massive expansion and acquire effector function. The contraction phase is a death phase, where ~90% of the responding T cells die by apoptosis. The final phase involves development and maintenance of a long-lived population of memory T cells. Throughout these phases, CD8 T cells undergo the gradual differentiation and selection processes that eventually result in formation of effective CD8 T-cell memory. For instance, murine studies suggest that the duration of antigen stimulation [19–22] and the presence of CD4 T-cell help [23–25] during the initial immune response are critical for optimal CD8 T-cell memory development. In addition, murine CD8 T cells expressing high levels of the IL-7Rα chain (CD127) bypass the death phase, differentiating into memory T cells [26]. Long-term maintenance of murine memory T cells has been shown to be antigen-independent [27], relying on IL-7 and IL-15 signals for T-cell survival and homeostatic turnover respectively [26,28,29]. Similar studies of T-cell memory in both nonhuman primates and humans have only recently begun.

### Memory T-cell subsets

Memory T cells express molecules that are associated with migration (CCR7, CCR5, CD62L), cytotoxic potential (perforin, granzyme), activation status (CD38, HLA-DR, CD45RA/RO), susceptibility to apoptosis (Bcl-2) [30–32], and proliferation (Ki-67). Memory T cells can be divided into subsets exhibiting distinct phenotypic and functional properties. Effector memory T cells ($T_{EM}$: CCR7−, CD62L−) preferentially migrate to peripheral tissues, produce IFN-γ upon stimulation, and have direct *ex vivo* cytotoxicity. In contrast, central memory T cells ($T_{CM}$: CCR7+, CD62L+) are localized to lymph nodes and lack immediate effector function, but possess greater proliferative potential [33]. As previous murine studies found that $T_{CM}$ cells are more protective against viral challenge than $T_{EM}$ cells, alteration in those subsets could have important implications for how well protective immunity is maintained in immunosuppressed patients [34].

## Transplant patients have poor immune responses to vaccination

### General comments

While vaccination of healthy individuals decreases viral transmission and/or prevents disease, it is not known whether it has similar effects in the immunosuppressed. Large-scale studies of vaccination efficacy in transplant patients are lacking. Studies utilizing neutralizing antibodies as surrogate markers for successful vaccination do not necessarily correlate with effective protection from infection. Studies in immunosuppressed transplant patients demonstrate an attenuated response to certain vaccinations including influenza [35,36], hepatitis A [37], and diphtheria [38]; but not against pneumococcus [39] and the tetanus toxoid [38]. As protection from infection depends on the presence of neutralizing antibodies and cellular immune activity, inhibition of T-cell activity by conventional immunosuppressants may limit the predictive value of these otherwise protective antibody titers [40] (Fig. 1, dotted line). In spite of these limitations, some polysaccharide, peptide, toxoid, or killed vaccines are recommended, as potential benefits outweigh adverse effects (see Table 1) [41].

Whether more frequent vaccination would boost immunity remains unclear. In a small study of 16 heart transplant recipients, neutralizing antibodies to influenza vaccination were formed less commonly in immunosuppressed patients than in healthy controls (40–60% vs. 80–97%) [39] and booster vaccination at 4 weeks did not significantly increase vaccine efficiency [39]. In liver transplant recipients who generally receive less immunosuppression, primary influenza vaccination still led to production of less neutralizing antibodies than healthy controls (70% vs. 95%) [42]. A booster vaccination resulted in a small but significant increase in the number of liver recipients with neutralizing antibodies (70% vs. 80%) albeit with lower titers than controls [42].

### Influenza vaccination

Influenza infection is associated with higher morbidity, mortality, and organ rejection in renal transplant recipients [40]. In clinical trials, the theoretical risk of vaccination-induced rejection has not been observed [35,43]. Some studies in kidney transplant recipients demonstrate strain-specific defects in the development of neutralizing antibodies after vaccination [35,36,44], while others do not [45,46]. Most studies of other solid organ transplant recipients (heart, lung, liver) also show strain-specific differences in vaccine efficiency [39,46,47]. Thus, some influenza strains may be more immunogenic than others. Alternatively, differences in the immunosuppressive

**Table 1.** Vaccines for transplant recipients.

| | | |
|---|---|---|
| Vaccines generally considered to be safe | | |
| Influenza | Inactivated trivalent | Yearly |
| Pneumococcal | Capsular polysaccharides | Every 5 years |
| Tetanus-diphtheria | Toxoid | Primary pretransplant. Boost every 5 years |
| *H. Influenza* | Polysaccharide | Primary pretransplant, Unknown booster |
| Hepatitis A | Inactivated whole | Primary pretransplant for liver patients or travelers to endemic areas, antibody titers helpful to determine responsiveness |
| Hepatitis B | Recombinant HBsAg | Pretransplant, antibody titers helpful |
| Polio | Inactivated trivalent | Primary pretransplant and use in household contacts |
| Controversial vaccines: use post-transplant indicated for high-risk populations | | |
| MMR | Live attenuated | Pretransplant recommended. Consider use post-transplant in nonimmune patients attempting pregnancy in areas endemic for rubella |
| Varicella | Live attenuated | Pretransplant recommended. Controversial use in nonimmune pediatric patients at high risk for primary varicella infection |
| Contraindicated vaccines: use by family members may place patient at risk | | |
| Oral polio | Live attenuated | Contraindicated. Avoid use in family members |
| Vaccinia | Live attenuated | Contraindicated. Avoid use in family members |
| Yellow fever | Live attenuated | Contraindicated. Avoid use in family members |

regimens employed may be responsible for the observed differences in vaccine efficiency [35,48].

Much less information is available on cell-mediated immune responses to influenza in vaccinated transplant recipients. Animal studies suggest that CD4 and CD8 T cells participate in clearing influenza infection [49], and suggest IFN-$\gamma$ production is crucial for clearance of a secondary influenza infection [50]. Lower neutralizing antibody levels in immunosuppressed patients may reflect an underlying defect in T-cell responsiveness, as efficient antibody production depends on T-cell help. In healthy humans, influenza vaccination leads to a viral strain-specific cellular response, as measured by cytokine and granzyme production. No systematic studies of T- and B-cell function following influenza vaccination have been conducted in humans receiving the state-of-the-art immunosuppression. A small study in lung transplant recipients revealed impaired virus-specific cellular responses as assayed by cytokine and granzyme B production, but did not correlate this cellular activity with neutralizing antibody production [47]. Another study in liver transplant recipients that attempted to correlate virus-specific PBMC proliferation with neutralizing antibody production showed only a nonstatistically significant trend towards correlation [42]. In a recent study, Ballet *et al.* compared influenza vaccination in immunosuppressed renal transplant patients and patients having stable renal function years after cessation of immunosuppressive treatment. In this study, while patients under conventional immunosuppressive treatment showed a weak humoral and T-cell response compared with healthy volunteers, three of five patients free of all immunosuppressive therapy demonstrated immune response comparable with healthy volunteers [51].

It is important to establish whether there is a causal link between cell-mediated responses, neutralizing antibody production, and overall protection against viral infection to understand fully the effects that different immunosuppressive regimens may have on the potential for vaccine efficiency.

### Smallpox vaccination

Smallpox belongs to the genus Orthopoxvirus, family Poxviridae, which includes the variola (smallpox), vaccinia and monkeypox viruses. Although presently not occurring naturally, infection by smallpox is known to be highly communicable, spread rapidly, and be associated with a mortality approximating 30% that could approach 100% in immunosuppressed patients [52]. The smallpox vaccine utilizes an attenuated live vaccinia virus; as the Poxviridae family viruses are highly similar, cross-protection occurs after infection or 'vaccination'. This vaccination results in viral replication, establishment of anti-viral immunity, and long-term protection against smallpox [53–57].

The 'Dryvax' smallpox vaccine contains live vaccinia virus isolated from the lymph of calves infected with the New York City Board of Health strain [58]. About 10 days after inoculation in humans, neutralizing antibodies can be detected in the blood [56]. Two weeks after vaccination, vaccinia-specific CD4 and CD8 effector T cells are identifiable in antigen stimulation assays [59]. Formation of a pool of memory CD8 T cells [53] that declines slowly over several decades follows [11,12]. In contrast, virus-specific memory B cells and antibody titers continue to remain stable in the absence of antigenic re-exposure [11,12].

Studies using the smallpox vaccine in solid organ transplant recipients have not been performed as patients with defects in T-cell immunity may develop severe life-threatening complications resulting from progressive vaccinia infection after inoculation with smallpox vaccine [60]. Progressive infection by either vaccinia necrosum or vaccinia gangrenosum poses a major risk for immunosuppressed persons with progression at the primary vaccination site and viremic spread to other sites over a period of days or weeks [61]. Obviously, a safer vaccination for smallpox would be needed for immunocompromised individuals. One possibility is to use the modified vaccinia Ankara strain (MVA) which after more than 500 passages of vaccinia in chick embryo fibroblasts acquired multiple gene mutations that resulted in the loss of its ability to replicate effectively in human cells [62]. As the vaccines to prevent smallpox cannot ethically be tested in humans, this approach has been tested in nonhuman primates. In healthy nonhuman primates, two doses of MVA proved as effective as the licensed Dryvax vaccinia vaccine in protecting against infection by monkeypox. Immunization produced neutralizing antibodies and specific T-cell responses, detectable as early as 1 week postvaccination and sustained for over 16 weeks [63]. In a 13-day study of immunosuppressed (lymphocyte depleted or total body irradiated) nonhuman primates, MVA vaccination was safe and effective, as determined by production of a vaccinia virus-specific antibody response [64]. Because organ transplant recipients require immunosuppression for life, live vaccines such as MVA need to be carefully tested for both long-term safety and efficacy in immunosuppressed nonhuman primates prior to study in human transplant recipients. While smallpox is considered eradicated worldwide and arguably only two well-protected stockpiles exist, there is the threat that it could be reintroduced by terrorists into the environment. Also, there have been a few natural outbreaks of less severe monkeypox in humans, which could also pose a risk to immunosuppressed patients. The threat that smallpox or even monkeypox could be re-introduced to the population by bioterrorists mandates studies to understand the effects of the MVA vaccine on immunosuppressed populations and determine its potential as a vaccine in this group.

### Other vaccinations and transplantation

Earlier reviews on vaccination of transplant recipients have raised issues that merit discussion [65,66]. While some vaccines, such as influenza, lead to a poor antibody response in transplant patients compared with healthy subjects, other vaccines, such as pneumococcus, do not lead to an impaired response [39]. It has been hypothesized that polysaccharide vaccinations may be more effective in transplant recipients than peptide-based vaccinations because of their lack of dependence on T-cell help.

The immune system maintains immunologic memory of a viral pathogen either to prevent re-infection or to clear an infection more rapidly. However, there is evidence that maintenance of immunologic memory is impaired in immunosuppressed transplant recipients. For example, liver and kidney transplant recipients usually produce protective antibody titers in response to immunization against hepatitis A similar to healthy subjects; however, they fail to maintain this response with only 59% of liver and 26% of kidney transplant recipients maintaining protective antibody titers at 2 years compared with 100% of healthy subjects [37]. Similar findings were reported for the diphtheria vaccine, but not polio [38] or pneumococcus [67]. Recently, two independent studies evaluated hepatitis B surface antigen–specific antibody titer in response to hepatitis B vaccination in liver transplant recipients and found that hepatitis B immunoglobulin prophylaxis was not required in a majority of the patients [68,69]. Furthermore, in these studies, vaccination was effective against *de novo* hepatitis B infection [68] and did not have any side effects [69].

In contrast to the list of vaccines that are generally considered to be safe and effective based on a large number of clinical studies, the use of certain live-attenuated vaccines in solid organ transplant population is controversial (Table 1). Studies using live-attenuated vaccines [varicella and MMR (mumps, measles, rubella)] have been performed mostly in pediatric transplant recipients. With varicella vaccine, even though vaccination in pediatric solid organ transplant recipients was effective in reducing the incidence of disease [70], concerns about the reactivation of the vaccine strain in the immunosuppressed population and the possibility of spread and onset of zoster in the general population warrants further clinical trials before the vaccine is considered safe for use in solid organ transplant recipients [71]. Despite some studies in bone marrow transplants and pediatric liver recipients [72], MMR, another live-attenuated vaccine, has not been deemed necessary in solid organ transplants as rubella infection does not cause severe disease in solid-organ recipients, and also because of the fact that the efficacy and safety have not been established in adult solid-organ recipients. Furthermore, some live-attenuated vaccines such as oral polio, vaccinia, and yellow fever are regarded as contraindicated vaccines for transplant recipients since no data are available [73]. Moreover, oral polio vaccine carries the potential for shedding of the virus and the possibility of transmission to family members of transplant recipients.

 

## Immunosuppressive drugs: distinct mechanisms of action, differing immunodeficiencies

### General overview

At present, a successful outcome in transplantation is dependent on the prevention of graft rejection through life-long therapy with immunosuppressive drugs. The principal targets of immunosuppressive therapies are either T cells or antigen presenting cell (APC) signals leading to T-cell activation (reviewed in [74]). The three-signal model of the T-cell activation (Fig. 2) can be used to describe the mechanism of action of different immunosuppressive drugs. The primary antigen-induced signal (Signal one) is received by the TCR/CD3 complex. Signal two, delivered via costimulatory molecules, amplifies the signal one resulting in cytokine production and induction of anti-apoptotic factors. Signal three results from the engagement of cytokine receptor, particularly those specific for cytokines using the common gamma chain (IL-2, IL-7, and IL-15). Engagement of cytokine receptors results in signals transduced via JAK3 and mTOR that contribute to T-cell proliferation. As individual immunosuppressive drugs inhibit different pathways involved in T cell and B-cell activation (Fig. 2), it is likely that they have differential effects on the immune response to vaccination. To understand better these potential differences, the known mechanisms of action of both commonly used immunosuppressants and novel immunosuppressive agents are detailed below.

### The calcineurin-inhibitors

Cyclosporin and tacrolimus are CNIs that bind to cytosolic proteins to inhibit calcineurin-phosphatase. This inhibition prevents the nuclear translocation of NFAT, a transcription factor, in response to TCR/CD3 signals to prevent activation of lymphocytes. Although remarkably effective at preventing graft rejection, they undoubtedly

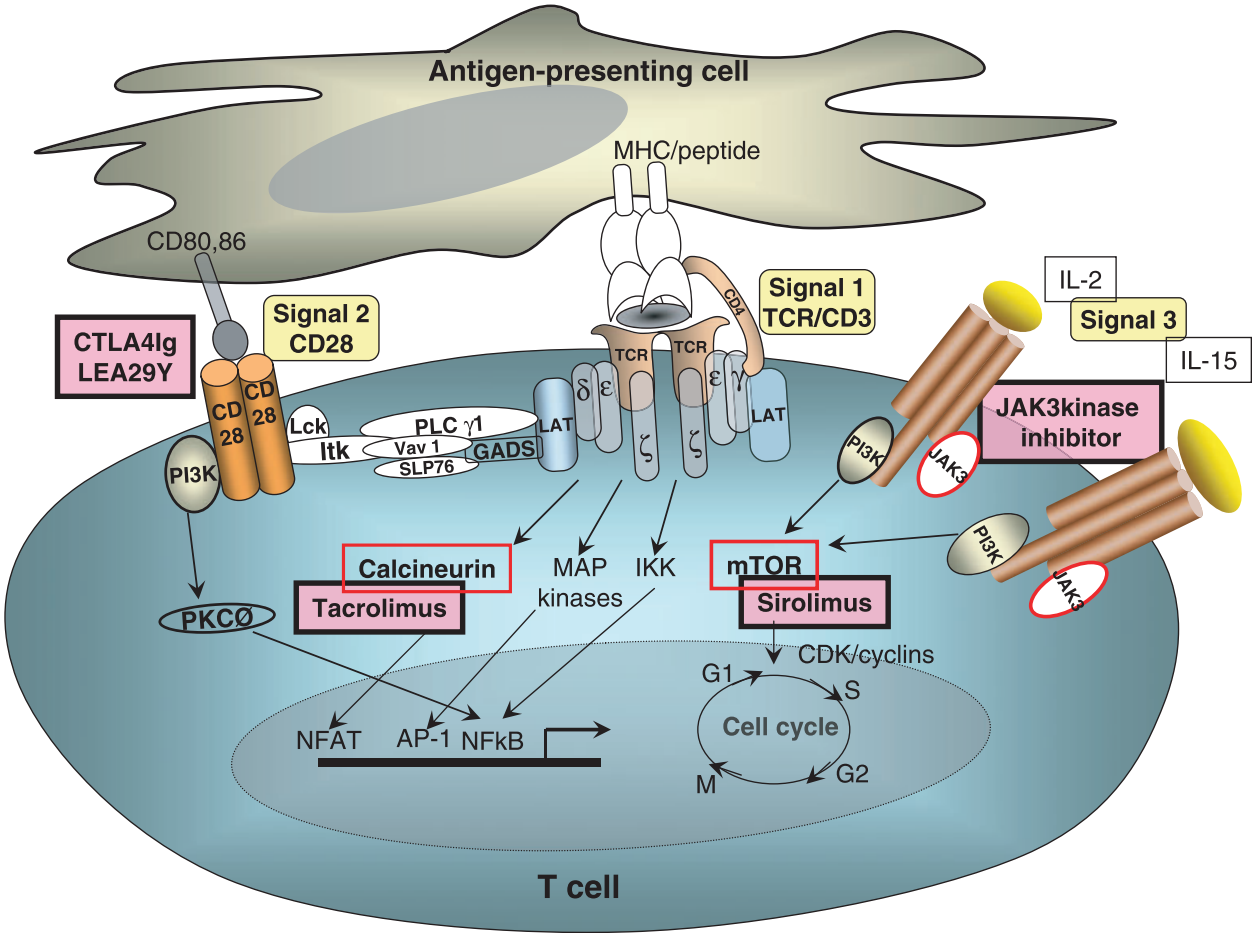


**Figure 2** Schematic representation of the signaling pathways inhibited by commonly used immunosuppressants and novel immunomodulatory agents. Key interactions between an antigen presenting cell and a responding T cell are shown.


 

contribute to the defective vaccine responses observed in transplant patients. The effects of continuous CNI therapy on the human innate and adaptive immune systems are not known. Without this fundamental knowledge, it is difficult to design rational strategies to protect patients who require treatment with CNI from specific pathogens.

Because of their impact on early antigen-dependent T-cell activation, CNIs affect the magnitude of the T-cell expansion phase. Additionally, TCR signal attenuation by CNIs may alter the TCR-self MHC interactions required for survival of naïve T cells in the periphery [27] thereby affecting the longevity and perhaps the repertoire of naïve T cells emigrating from the thymus. In younger recipients, this effect may not significantly impair immunity, but the effects of the drug could be more significant as thymic function declines with age. CNIs may also impair vaccination efficiency because of their potent inhibitory effects on the maturation and activation of DCs [75].

### Anti-proliferative agents (azathioprine and mycophenolic acid)

Azathioprine, a prodrug releasing 6-mercaptopurine, ultimately inhibits DNA synthesis and inhibits both T and B-cell proliferation in addition to other rapidly dividing cell types. Recent information suggests azathioprine induces T-cell apoptosis through blockade of CD28-mediated Rac1 activation [76].

Mycophenolic acid, the active component of mycophenolate mofetil and mycophenolate sodium, inhibits inosine monophosphate dehydrogenase activity, preventing guanosine nucleotide synthesis in lymphocytes and other cells lacking the salvage pathway of purine biosynthesis. In animal studies, it inhibits antibody production in a T-cell independent manner [35], which could affect vaccine efficacy. In this regard, the limited studies performed to date are contradictory [35,36].

### Corticosteroids

The broad anti-inflammatory effects of corticosteroids are still not fully defined. These drugs bind glucocorticoid receptors and once translocated to the nucleus, reduce production of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IFN-$\alpha$, and TNF-$\alpha$ [77]. Glucocorticoid immunosuppression alone appears not to alter vaccine efficacy [66].

### mTOR inhibitors

Sirolimus and everolimus are increasingly used in the postrenal transplant maintenance phase. These agents act to limit lymphocyte proliferation by inhibiting mTOR, a signaling molecules engaged when cytokine receptors that use the common cytokine receptor gamma chain are bound by their cytokine ligands [74]. Two cytokines/cytokine receptors that use the common gamma chain, IL-7 and IL-15, are crucial for initiating the survival and long-term cell cycling of memory T cells, respectively [26,28]. IL-15-deficient mice cannot mount a protective immunologic response to vaccinia virus infection [29]. Additionally, some mTOR inhibitors inhibit murine DC development and expansion [75].

### New immunosuppressive agents and emerging drugs

It is critical to study the currently approved regimens mentioned above. However, a number of promising new immunosuppressive agents are currently being evaluated in transplant recipients. In as much as many of these have unique mechanisms of action, they will require independent study. These agents primarily target T cells (ATG) [78], B cells (anti-CD20 antibody) [79], T cells and B cells (anti-CD52 antibody; alemtuzumab) [80], and cytokine receptor on T cells (anti-IL-2 receptor antibody; basiliximab and daclizumab) [81]. However, recent studies have suggested that the effects of some of these reagents extend beyond intended use; for example, ATG has the potential to suppress DCs [82] and both ATG and alemtuzumab also deplete NK cells [83]. As some of these agents are shown to influence other subsets of T cells with potential regulatory function ($CD4^+$ $CD25^+$ T cells) [84], it is critical to understand the effects of this new class of immunosuppressive agents on immune responses to vaccination, particularly with respect to safety.

While a number of well-designed clinical trials support the evaluation of some of the above mentioned immunotherapeutic strategies for vaccine efficacy studies, two strategies that exploit T-cell activation signaling cascade have shown significant promise in preclinical animal models and clinical trials of solid organ transplantation. First, as discussed, complete T-cell activation requires costimulatory signal by receptors such as CD28. B7 family receptors B7-1 (CD80) and B7-2 (CD86), expressed by APCs, are ligands that can bind to both CD28 and its homologue CTLA4 (Fig. 2). CTLA4-Ig is a soluble recombinant immunoglobulin fusion protein, comprises the extracellular portion of CTLA4 and the constant portion of an IgG1 antibody. Recently, a high-affinity mutant derivative of CTLA4-Ig, LEA29Y (belatacept), was shown to inhibit very effectively activation of human T cells. Phase III clinical trials in renal transplantation are now underway [85]. Companion studies may begin to define the effects of LEA29Y on the response to vaccines. Second, a JAK3 inhibitor (CP-690550) showing immunosup-

 

pressive properties in nonhuman primate transplant models is under clinical development for use in renal transplantation [86]. Cytokine receptors that use the common γ-chain (IL-2, IL-4, IL-7, IL-9, IL-15, IL-21) require the cytoplasmic tyrosine kinase JAK3 for signaling critical for the development and homeostasis of immune cells. Patients with mutations in common γ-chain suffer from severe combined immunodeficiency (SCID) [87], and those lacking JAK3 expression also display a SCID phenotype [88]. Studies in nonhuman primates revealed significant, dose-dependent decreases in circulating NK and $CD8^+$ T cells following administration of CP-690550 for 3 weeks, likely because of inhibition of IL-15 dependent homeostasis [89]. To date, no studies have addressed responses to infection or vaccination.

## Progress, current challenges, and unmet needs

To prevent graft rejection, those receiving transplants today must remain on immunosuppression for the rest of their lives. An increase in both success rates and the number of procedures has produced a growing population of immunosuppressed transplant recipients at a greater risk of infections, whether they originate naturally or as a result of bioterrorism. Additionally, little is known about the risks or efficacy of live, live attenuated, or killed vaccines in these patients. Despite the pressing need for more information, relatively few studies have been undertaken. In particular, studies must be designed that will determine the outcome of natural infections and vaccination in the setting of different immunosuppressive regimens. Identification of the specific innate and adaptive immune alterations and their clinical significance will aid in designing and choosing appropriate vaccines and anti-infective therapies, as well as in defining public health policy on prevention and treatment of the immunosuppressed during outbreaks.

Detailed mechanistic longitudinal studies of the effects of CNI- and sirolimus-based immunosuppressive regimens on adaptive and innate immunity in human renal transplant recipients have not been conducted. By collecting serial blood samples from recipients who have or have not received vaccinations (most likely the inactivated influenza vaccine) as well as from healthy controls, it should be possible to compare their adaptive and innate immune responses to the vaccine. As there are some limitations regarding immunizations and tissue collection (number of samples and types of tissue) in human studies, the vaccination regimens need to be tested in nonhuman primate models of transplantation. Additionally, such testing would be required for the study of responses to more harmful pathogens such as Poxvirus.

Beyond patient protection and health policy, the ramifications of these in-depth studies will, without doubt, advance the understanding of alloimmunity, pharmacological development of immunosuppressive drugs, vaccination strategies, and ultimately contribute to a more thorough understanding of those factors necessary for the development and maintenance of immunologic memory. Thus, research on immune response and protection will not only touch the immunosuppressed patient community, but extend to future patients and the well-being of the general population.

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