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From the classic concepts to modern practice

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Abstract

Transplant infectious disease is a field in evolution. For most allograft recipients, immunosuppressive therapies are more potent and have reduced the incidence of acute allograft rejection. At the same time, these therapies have increased susceptibility to many opportunistic infections and virally-mediated malignancies. Immunological tolerance has been achieved in only small numbers of patients who avoid drug toxicities and infection for as long as tolerance persists. The traditional timeline of post-transplant infections remains useful in the development of a differential diagnosis for patients with infectious syndromes. However, patterns of infection in the post-transplant period have changed over the past decade. Recipients are derived from a broader range of socioeconomic and geographical backgrounds. Infections are diagnosed more often, with improved microbiological assays (e.g. nucleic acid testing, NAT) used routinely in the diagnosis and management of common infections and increasingly in the screening of organ donors. Patterns of opportunistic infection have been altered by the increased identification of organisms demonstrating antimicrobial resistance and by the broader use of strategies to prevent viral, bacterial and fungal (including *Pneumocystis*) infections. Newer techniques are being applied (e.g. HLA-linked tetramer binding, intracellular cytokine staining) to assess pathogen-specific immunity. These are being integrated into clinical practice to assess individual susceptibility to specific infections. Infection, inflammation and the human microbiome are recognized as playing a central role in shaping innate and adaptive immune responses, graft rejection and autoimmunity. The full impact of infection on transplantation is only beginning to be appreciated.

Keywords: organ transplantation, donor-derived infection, viral infection, immunosuppressive therapies, microbiome, nucleic acid test, opportunistic infections, prophylaxis, solid organ transplantation, tolerance

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Hot Topics

- Consider alterations in the timeline for infection with institution-specific strategies for immunosuppression and prophylaxis.
- Evaluate the cost-effectiveness of newer immunosuppressive regimens and laboratory assays.
- In resource-limited regions, which forms of immunosuppression and prophylaxis are cost-effective?
- The impacts of changes in the human microbiome, vaccination and antimicrobial therapies on graft survival are poorly understood. Consider interactions of specific pathogens with the innate and adaptive immune systems on graft function.

General Principles: the Risk of Infection after Transplantation

The diagnosis of infection is more difficult in transplant recipients than in immunologically normal hosts due to the effects of immunosuppression, which obscures the signs and symptoms of infection both acutely (inflammation) and chronically (cellular infiltration) [1–3]. Clinical presentations are

often complicated by non-infectious causes of fever (e.g. graft rejection). Drug toxicities and drug interactions (e.g. azole anti-fungal agents with calcineurin inhibitors) are common. Multiple simultaneous processes are often present (e.g. graft rejection and infection). As a result, specific microbiological and immunological diagnoses are needed to optimize therapy; invasive diagnostic procedures are often needed to achieve timely diagnoses.

One of the general principles of transplant infectious disease is that the *prevention of invasive disease, whether resulting from new exposure or by the activation of existing, latent infection, is easier than the treatment of established disease*. True toxicity of prophylaxis with low-dose antivirals, antifungals or daily trimethoprim-sulphamethoxazole (TMP-SMZ) is uncommon, although commonly misdiagnosed [1]. Toxicity of the treatment of such infections is common and may be life-threatening or cause permanent graft injury. In the absence of assays that allow individualization of immunosuppression after transplantation, prophylactic strategies are based on an assessment of the anticipated risk of infection based on experience (e.g. about 15% incidence of *Pneumocystis* pneumonia in immunosuppressed hosts without prophylaxis) or based on the ability to stratify risk based on serological or microbiological testing, epidemiological history, and the perceived intensity of immunosuppression. Thus, organ recipients who are colonized with VRE or *Aspergillus* or who receive seropositive organs for cytomegalovirus (CMV) or Epstein-Barr virus (EBV) require different prophylaxis and/or monitoring at different phases of the transplant continuum than those who lack such exposures. *The risk of infection is a continuous function of the interplay between these factors.*

Epidemiological exposures

Epidemiological exposures can be divided into four overlapping categories: donor- and recipient-derived infections, and community or nosocomial exposures.

Donor-derived infections. Infection is commonly transmitted with donor organs in the form of latent viral infections of the graft (e.g. CMV and EBV), infection or unrecognized colonization of the lungs, unknown bacteraemia or urinary tract infections, or surgical contamination at procurement or preservation. Infected organ donors have been found to transmit bacteria and fungi carrying resistance to routine surgical antimicrobial prophylaxis [4]. In the past few years, unexpected clusters of donor-derived infections in transplant recipients have been recognized, including those due to West Nile virus, lymphocytic choriomeningitis virus (LCMV), rabies, HIV, hepatitis B and hepatitis C viruses, herpes simplex virus, tuberculosis, endemic fungi and Chagas' disease [4–8]. Controversy persists regarding the use of organs from donors with undefined clinical syndromes (e.g. 'altered mental state' or fever), which have had a disproportionate role in the transmission of unusual pathogens associated with central nervous system infection or bacteraemia. This effect is amplified by the shortage of donor organs and the limited time-frame in which microbiological screening must be performed. These observations illustrate the need for new approaches to microbiological screening of donors.

Active or latent infections in transplant recipients should be eradicated or controlled to the greatest degree possible prior to transplantation as these will be exacerbated by immunosuppression [8]. Common recipient-derived pathogens include *M. tuberculosis*, some parasites (*Strongyloides stercoralis* and *T. cruzi)*, viral infections (herpes simplex virus (HSV) or varicella zoster virus (VZV, shingles)), endemic fungi (*Histoplasma capsulatum*, *Coccidioidioides immitis* and *Paracoccidioides braziliensis*), hepatitis B or C or, more recently, HIV. Although previously contraindicated, successful organ transplantation has been achieved in HIV-infected patients treated with highly active antiretroviral therapy (HAART), and in some cases with HIV-infected organ donors [9,10]. Employment, hobbies, travel, pets or marijuana use (*Aspergillus* species) may suggest clinically important exposures.

Net state of immunosuppression

The concept of the 'net state of immunosuppression' comprises all factors that may contribute to the risk of infection (Table 1) [1–3]. The impacts of preexisting disease processes are often underestimated. Renal failure and dialysis are associated with poor responses to bacterial infections and colonization with hospital-acquired flora [11]. Cirrhosis and portal hypertension reduce acute inflammatory responses (specific antibody formation, chemotaxis) and predispose to infection caused by *Cryptococcus* and *Aspergillus* species [12,13]. Lung failure may be associated with bacterial and fungal colonization and poor microbial clearance. These infectious hazards must be added to the post-transplant effects of immunosuppressive therapy (Table 2). The effects of some of

TABLE 1. The 'net state of immune deficiency'

Preexisting immune deficits
Critical illness
Malnutrition
Organ dysfunction (uraemia, cirrhosis, COPD/cystic fibrosis, heart failure)
Diabetes
Colonization with antimicrobial-resistant pathogens, hospitalization
Immunosuppressive therapies (current and past)
Acquired immune deficiencies (e.g. hypogammaglobulinaemia)
Prior therapies (chemotherapy, antimicrobials)
Mucocutaneous barrier integrity (catheters, lines, drains)
Fluid collections (blood, lymph, urine, bile, pus)
Neutropenia, lymphopenia
Viral co-infection (e.g. CMV, EBV, HCV, HBV, HIV)

TABLE 2. Risk assessment in transplantation

Greater infectious risk
Critical illness entering transplantation
Prior colonization with antimicrobial-resistant pathogens
Induction therapy—lymphocyte depletion
High-dose corticosteroids
Plasmapheresis (not well studied)
High rejection risk (HLA mismatch desensitization)
Early graft rejection
Graft dysfunction
Technical complications
Anastamotic leak
Bleeding
Wound infection/poor wound healing
Prolonged intubation/intensive unit care
Surgical, vascular or urinary catheters
Lower infectious risk
Immunological tolerance
Good HLA match
Technically successful surgery
Good graft function
Appropriate surgical prophylaxis
Effective antiviral prophylaxis
PCP prophylaxis
Appropriate vaccination

these therapies such as the biological agents (induction therapy via lymphocyte depletion) are only beginning to be understood in terms of the repertoire of immune specificities achieved during immune reconstitution [14]. Multiple mechanisms of tolerance (e.g. central vs. peripheral deletion or anergy) have been demonstrated in patients with induced or spontaneous immunological graft tolerance. Some gaps in function (e.g. NK cells, antiviral immunity) persist for months to years. Breeches in mucocutaneous integrity (e.g. vascular and urinary catheters) and fluid collections (hematoma, ascites and effusions) are magnets for microbial seeding.

Prevention of Infection

Antimicrobial prophylaxis has significantly altered the incidence and severity of post-transplant infections. Six general preventive strategies are used: (i) vaccination, (ii) surgical prophylaxis, (iii) universal prophylaxis, (iv) preemptive or presymptomatic therapy, (v) 'targeted prophylaxis' and (vi) educated avoidance. 'Universal prophylaxis' provides antimicrobial therapy to all 'at-risk' patients for a defined time period. 'Preemptive therapy' utilizes a sensitive, quantitative assay (e.g. molecular, antigen detection) to monitor patients for the presence of a specific disease at predetermined intervals to detect early infection prior to the emergence of invasive disease. Positive assays initiate therapy. Thus, the term 'presymptomatic' might be better employed for these interventions. 'Targeted prophylaxis' is a new term for the use of assays that make possible assessment of the individual's susceptibility to specific pathogens (i.e. prophylaxis in patients at risk of infection and lacking immunity against that pathogen based on laboratory assays). Individuals considered to be over-immunosuppressed based on qualitative assays or intensification of immunosuppression (e.g. for graft rejection) should have their primary prophylaxis reinstituted. 'Educated avoidance' includes lifestyle changes that may limit exposure to potential pathogens (wearing masks or gloves while gardening, avoiding attics or basements with moulds, and using filtered water supplies). Preemptive therapy incurs extra costs for monitoring and coordination of outpatient care while reducing drug costs and drug toxicities. These are discussed elsewhere in regard to anti-CMV therapies. Routine surgical prophylaxis should be adjusted to the organ transplanted and individual exposures or colonization patterns and hospital epidemiology. Surgical prophylaxis may be adjusted based on known colonization patterns with organisms such as *Pseudomonas*, MRSA, VRE or fungi.

Two advances in prophylaxis have significantly altered transplant medicine. First, trimethoprim-sulphamethoxazole (TMP-SMZ) is given at most centres for 3 months to a lifetime to prevent *Pneumocystis* pneumonia (PCP) as well as *Toxoplasma gondii*, *Isospora belli*, *Cyclospora cayetanensis*, many *Nocardia* and *Listeria* species, and common urinary, respiratory and gastrointestinal pathogens. Low-dose TMP-SMZ is well tolerated and should be used in the absence of specific data demonstrating allergy or interstitial nephritis. Alternative anti-*Pneumocystis* prophylactic strategies lack this breadth of protection [3,15]. The prevention of post-transplant cytomegalovirus and other herpesvirus infections, including the availability of some oral antiviral agents and the use of nucleic-acid-based assays to establish a specific microbiological diagnosis and to monitor responses to therapy for many viral infections, have also revolutionized post-transplant care. These are discussed in detail elsewhere [3].

The Timeline of Infection

The timeline of post-transplant infections reflects the post-transplantation relationship between the recipient's epidemiological exposures and immunosuppressive strategy employed. The timeline is used to establish a differential diagnosis for infectious syndromes at various stages after transplantation. Infections occurring outside the usual period or of unusual severity suggest excessive immunosuppression or epidemiologic hazard. *The timeline is 'reset' to the period of greatest risk for opportunistic infection with the treatment of graft rejection or intensification of immune suppression (e.g. bolus corticosteroids or T-cell depletion).* Changes in immunosuppressive regimens, routine prophylaxis and improved graft survival have altered the timeline somewhat. Initial immunosuppression is evolving from standard 'triple immunosuppression' (predni-

sone, calcineurin inhibitor and an antimetabolite such as mycophenylate mofetil; Fig. 1) to a variety of 'induction' regimens (T-cell depletion and co-stimulatory blockade) with calcineurin inhibition and/or mTor inhibition, often with an antimetabolite [1–3]. The use of induction therapy, notably with T-cell depletion, requires careful attention to CMV prophylaxis. Steroid-sparing regimens and anti-*Pneumocystis* prophylaxis have made PCP less common. Herpesvirus infections are uncommon during antiviral prophylaxis. Lymphocyte-depleting therapies produce prolonged T- and B-cell deficits and may alter T-regulatory subsets, antibody production and dendritic and NK cell functions. The long-term impacts of these agents and of the inhibitors of co-stimulatory T-cell pathways include a prolonged risk of (late, post-prophylaxis) viral and fungal infections and increased risk of post-transplant lymphoproliferative disorders (PTLD) and other malignancies. Recent application of antibody depletion (plasmaphersis), bortezimib and splenectomy in desensitization protocols diminish opsonization of bacteria and yeasts and have increased the risk of infection (e.g. encapsulated organisms and yeasts). Sirolimus-based regimens have been associated with poor wound healing and peripheral oedema, and with a form of non-infectious pneumonitis easily confused with PCP or viral pneumonia [16].

Phase 1: early post-transplantation (1–4 weeks)

Opportunistic infections are generally absent in the first month after transplantation as the full impact of immunosuppression depends on prolonged exposure to suppressive therapies. Infections in this period are generally donor or recipient derived (colonization, viraemia and candidaemia) or associated with technical complications of surgery (e.g. infected haematoma and peritonitis). Unexplained early infectious syndromes (hepatitis, pneumonitis, encephalitis, rashes and leucopenia) reflect donor-derived infection. *C. difficile* colitis is common. Early graft injuries (e.g. ischaemia to bile ducts or pulmonary reperfusion injury) may manifest later as foci for liver or lung abscesses (Fig. 1).

Phase 2: 1–6 months post-transplantation

In this period, TMP-SMZ prophylaxis should prevent most urinary tract infections and opportunistic infections such as

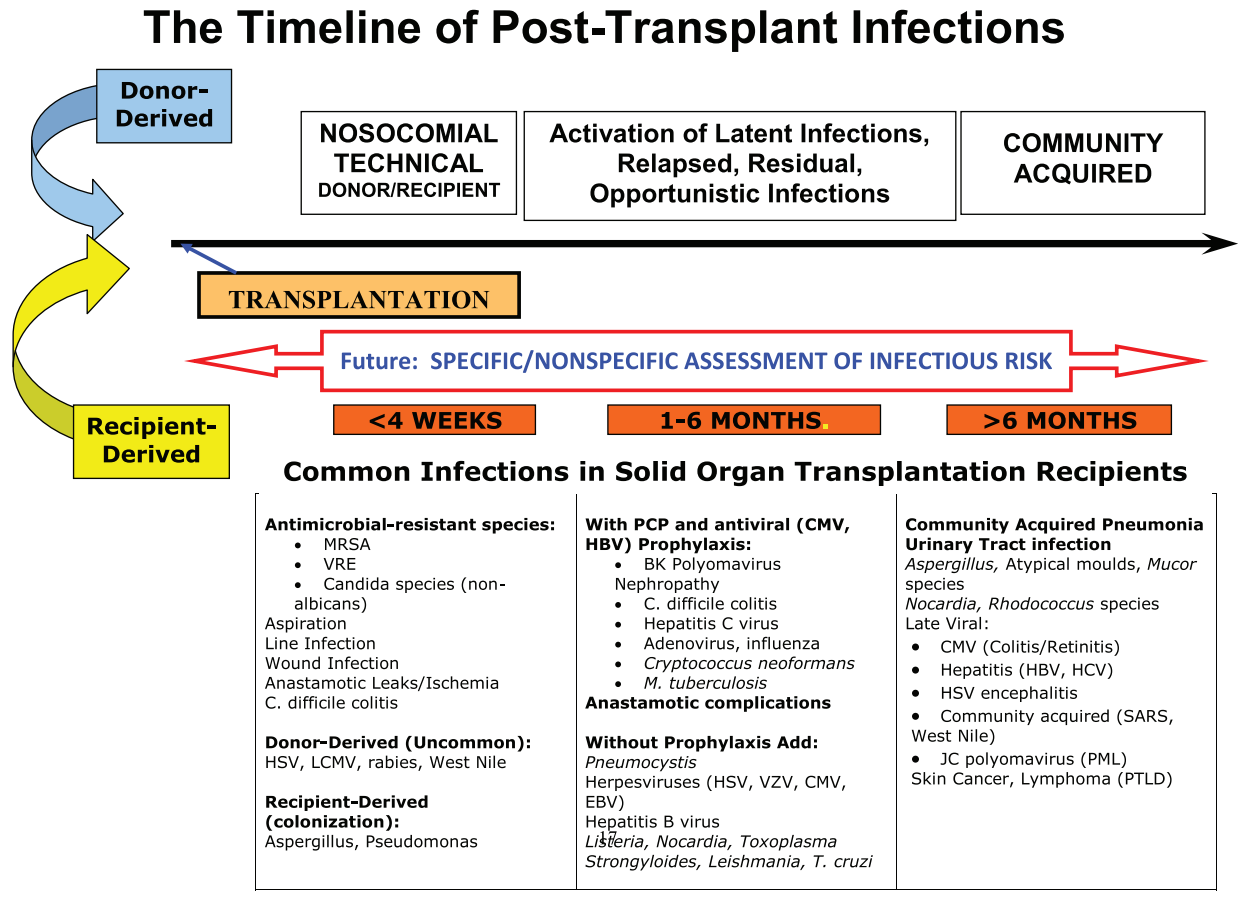

FIG. 1. The timeline of post-transplant infections. Redrawn from refs [1–3].

PCP, *L. monocytogenes*, *T. gondii* and sulfa-susceptible *Nocardia* species. Some infections (cholangitis, pneumonia and *C. difficile* colitis) persist from the perioperative period. Viral pathogens and graft rejection are responsible for the majority of febrile episodes in this period. Herpesvirus infections are uncommon in the face of antiviral prophylaxis but often emerge subsequently. Other viral pathogens, including BK polyomavirus, adenovirus and recurrent hepatitis C virus (HCV), reflect the patient population and intensity of immunosuppression. Among infections reactivated during this period are the endemic fungi, *Aspergillus species*, *Cryptococcus neoformans*, *Toxoplasma gondii*, *Trypanosoma cruzi* and *Strongyloides*.

In the past, we have taught that viral infections may cause immediate or 'direct' (tissue invasive) disease or may cause an array of virus-associated phenomena loosely termed 'indirect effects'. These include systemic (CMV) or local (influenza) immune suppression predisposing to or enhancing other opportunistic infections or PTLD and an increased risk of acute and chronic graft injury or rejection. *A significant body of data suggests that 'indirect effects' might be better termed 'microbially-determined immune modulation (MDIM).'*

- The *microbiome* has been redefined in terms of organisms living synergistically with the host (all forms including bacteria, viruses, fungi and parasites) and determining some aspects of host immune function. Acute, latent and chronic infections participate in these effects. *Pertubation or activation of the microbiome* has pleotropic effects in terms of infectious risk (colonization patterns) and immune function (antigen specificity) [17–19].
- *Heterologous immunity* is immune 'memory' of previously encountered pathogens, which alters subsequent immune responses to unrelated pathogens or grafts. Thus, prior antigenic exposures may provoke graft rejection or modulate responses to subsequent infections. This may also suggest that infectious exposures including vaccination will have both beneficial and detrimental effects in terms of allogeneic immunity.
- Organisms are also 'designed' (often genetic traits co-evolved with the human host) to avoid detection or attack by innate or adaptive immune functions. Thus, CMV has a genome of 235 Kbp, encoding over 165 genes and over 70 viral proteins. Other than the genes required for viral replication, many of the known gene products affect the host cell or immune response to the virus. Immune effects of parasites (Leshmania sp.) and fungal glycoproteins (glycans) and their receptors are being described.
- The *innate immune system* is increasingly recognized as being essential to the activation and specificity of adaptive immune functions. Interactions between microbial antigens and pattern-recognition receptors on monocyte/macrophages, dendritic cells and NK cells (e.g. Toll-like receptors and C-type lectin receptors) are essential to host defences and to the nature of the response to allogeneic (autoimmunity) and allogenetic (transplantation) antigens [20–24].

Phase 3: more than 6 months after transplantation

More than 6 months post-transplantation, infectious risk diminishes as immunosuppression is tapered in recipients with satisfactory allograft function. These patients tend to develop more severe manifestations of the common, community-acquired infections. Infection may occur in patients receiving intensified immunosuppression for graft rejection without prophylaxis. Chronic viral infections may contribute to graft injury (e.g. cirrhosis from HCV (livers), bronchiolitis obliterans (lungs) and accelerated vasculopathy (hearts) with CMV) or malignancy (PTLD, skin or anogenital cancers). This group will develop the side-effects of organ dysfunction. One group tends to have less adequate graft function over time, often receives more intensive immunosuppression and suffers recurrent infection despite subsequent attempts at immunosuppression minimization. These 'chronic ne'er-do-wells' are at increased risk of opportunistic infection with *Listeria* or *Nocardia* species, invasive fungal pathogens (*Zygomycetes* and *dematiacious* moulds) and unusual organisms (e.g. *Rhodococcus* species). Minimal signs of infection merit careful evaluation in such 'high-risk' individuals. They may benefit from lifetime TMP-SMZ or antifungal prophylaxis.

Future Directions

Given improved immunosuppression, the prevention of infection has become a cornerstone of modern transplantation. Individualization of prophylaxis and immunosuppression will require incorporation of individual factors (genomics, pharmacogenomics and proteomics) and advanced assays to assess graft- and pathogen-specific and non-specific measures of cell-mediated 'immune function'. Multicentre cohorts of transplant recipients will be required to study the diagnosis and management of relatively infrequent events such as virally-mediated malignancies or the role of infection in graft rejection. Better understanding of the role of the innate immune system may allow use of a lower intensity of 'global' immunosuppression. Tolerance strategies are advancing. Vaccines for CMV and other pathogens will alter post-transplant prophylactic strategies. Improved microbiological diagnostic tools (e.g. multiplexed assays using a variety of diagnostic

modalities) will improve donor screening and the diagnosis and management of invasive infections in transplant recipients. Investigation of the pathophysiological mechanisms underlying the pleotropic effects of infection, which predispose the patient to opportunistic infection and malignancy and graft rejection, are needed. The role of the microbiome and innate immunity are beginning to be explored in terms of control of immune function and specificities [17–24]. More judicious use of antimicrobial agents and microbial reconstitution may guide clinical practice in the future.

Transparency Declaration

The author has no conflicts of interest related to the material presented.

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