Screening and Monitoring for Infectious Complications When Immunosuppressive Agents Are Studied in the Treatment of Autoimmune Disorders

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Significant progress has been made in the development, investigation, and clinical application of immunosuppressive agents to treat a variety of autoimmune disorders. The expansion of clinical applications of these new agents requires the performance of large multicenter clinical trials. These large clinical trials are particularly important as one considers these agents for the treatment of type 1 diabetes, which although autoimmune in its pathogenesis, is not classically treated as an autoimmune disorder. Although these agents hold promise for amelioration or cure of this disease, they have the potential to facilitate infectious complications. There are limited data regarding the prospective assessment of infectious risks with these agents in trials of this nature. Pediatric subjects may be at greater risk due to the higher likelihood of primary infection. A subgroup of experts associated with TrialNet (a National Institutes of Health [NIH]-funded Type 1 diabetes mellitus research network) with expertise in infectious diseases, immunology, and diagnostics developed an approach for screening and monitoring of immunosuppression-associated infections for prospective use in clinical trials. The goals of these recommendations are to provide a structured approach to monitor for infections, to identify specific laboratory testing and surveillance methods, and to consider therapies for treatment of these potential complications. Prospective evaluations of these infectious risks allow for greater scientific rigor in the evaluation of risk, which must be balanced with the potential benefits of these therapies. Our experience supports an important role for investigators with expertise in infections in immunocompromised individuals in protocol development of immunosuppressive trials in type 1 diabetes and potentially other autoimmune diseases.

Key words. clinical trials; immunosuppressive agents; infectious complications.

Immunosuppressive agents have the potential to impact favorably on a variety of immune-based diseases. These agents have been used in the management of a variety of conditions including immune thrombocytopenic purpura, autoimmune hemolytic anemia, juvenile idiopathic arthritis, rheumatoid arthritis, and dermatomyositis. More recently, there has been an increasing interest in exploring the use of immunosuppressive agents in the treatment of both patients with newly diagnosed type 1 diabetes mellitus and in at-risk subjects prior to the development of clinical diabetes in the hope of limiting the ongoing process of autoimmune beta cell destruction as well as the goal of disease prevention.

Although the potential of immunomodulation for treatment or prevention of diabetes is promising, this strategy involves the use of agents in patients not traditionally treated in this manner, thereby exposing them to new and as-yet-undefined risks. Children may experience primary infection with many pathogens [1–3] while enrolled in a clinical trial and on a study agent, potentially increasing the risk for more severe disease. Primary outcome data from clinical trials will provide information about the...
efficacy of these strategies; however, in clinical practice, patients and their physicians will need to make informed decisions balancing the risks and benefits associated with the use of these agents. Accordingly, there is a need to define the spectrum of risk factors for infectious complications during the development and evaluation of these approaches and potential mitigating strategies.

As part of the National Institutes of Health (NIH)-funded Type 1 Diabetes Clinical Research Network (TrialNet), an Infectious Diseases working group has been organized with the goal of providing a structured approach for assessing, mitigating, and documenting risk through the development and implementation of strategies for screening and monitoring of immunosuppression-associated infections for prospective use in clinical trials. Special consideration was given to pediatric patients because the onset of type 1 diabetes typically occurs in this age group. Therefore, children are preferentially enrolled in immunomodulating studies, because the time of autoimmune disease onset may be the critical window for therapeutic intervention, prior to the permanent consequences from the autoimmune process. The members of this group were selected because of their expertise with infections in immunocompromised patients (adult and pediatric), immunology, and diagnostics, and these members are actively involved in protocol review, development, and implementation of TrialNet studies. As part of our efforts, we have prepared the following recommendations as a guide to facilitate recognition of potential infectious complications likely to occur in these patients and to stimulate research efforts to define the incidence and magnitude of infectious sequelae in patients with autoimmune disease being treated with immunosuppressive regimens. These recommendations are based on literature review, clinical experience with other immune compromised populations (eg, solid-organ transplantation [SOT] and hematopoietic stem cell transplantation [HSCT], and those receiving the Food and Drug Administration-approved monoclonal antibody therapy such as adults with rheumatologic diseases), and experience acquired through clinical trials within TrialNet. These recommendations represent the expert opinion of the authors.

**Risk of Infection Associated With Use of Immunosuppressive Agents**

The use of immunosuppressive agents is associated with a variable risk of developing both more frequent and more severe infections. The infectious risk is a function of the potency, dose, intensity, and duration of the immunosuppressive therapy [4]. The risk may be greater in pediatric patients who often experience primary infections and suffer enhanced morbidity compared to adults in whom the presence of preexisting immunity may offer some degree of protection despite exposure to immunosuppression [5]. Immunosuppressive agents have different effects on the immune system, altering the susceptibility to different pathogens. Potent anti-T cell therapies may specifically increase the risk for *Pneumocystis jirovecii*, toxoplasmosis, and reactivation of herpesviruses. B cell-targeted interventions may impact (but not necessarily abrogate [6]) the efficacy of immunizations and increase the susceptibility to encapsulated bacterial and certain parasitic (eg, Babesia) [7]) infections but may also lead to reactivation of latent viruses such as hepatitis B virus [8–9]. Many immunosuppressive agents may have a mixed effect on T and B lymphocytes as well as on other components of the immune system and host defense (eg, skin and mucosal integrity) [10]. Furthermore, different immunosuppressive agents may have different durations of biologic effect [11].

The risk for infection must be considered in light of the overall condition, presence of comorbidities, and exposures of the host. There may be a differential risk of infection in patients with established type 1 diabetes compared with those who do not yet have overt clinical disease. In addition, subject behaviors and potential exposures should be carefully considered in relation to potential infectious risk: intravenous drug use (blood-borne pathogens); tattooing and body piercing (blood-borne pathogens and mycobacterial infection); and marijuana use (fungal infections). Because there are no easy-to-use algorithms to assess the various factors that may influence infectious disease risk, planning for and assessing the risk for infectious complications should be carried out on a protocol-specific basis in which (1) the predisposing risks associated with the primary disease under study and (2) the nature and duration of proposed immunosuppression are considered by physicians with experience and expertise in infections associated with these issues.

**PATHOGENS OF PRIMARY CONCERN**

Just as the consideration of the risk of infection must be individualized according to which immunosuppressant agent(s) are used, so too is there a need to evaluate the potential impact of different pathogens and their likelihood of causing clinically important diseases in research subjects participating in trials utilizing immunosuppressive agents. Potential pathogens can be categorized as follows: (1) common pathogenic chronic infections which can be minimized through the use of inclusion/exclusion criteria; (2) potentially important pathogens whose risk may be mini-
mized by monitoring; (3) other typical opportunistic or routinely encountered (eg, respiratory syncytial virus [RSV] or influenza) pathogens which are commonly seen in immunosuppressed individuals; and (4) pathogens of lesser concern either because they are less commonly seen or typically only cause disease in severely immunocompromised individuals (see online supplement). It is worth noting that concern for some potential pathogens may vary according to geographic region (eg, Histoplasma, Coccidioides) and may vary by season or from year to year (eg, influenza and West Nile Virus). Finally, the presence of acute symptomatic infection, although not necessarily excluding a subject from study participation, may require delay in enrollment until the infection has resolved.

**Pathogens With Potential Impact on Inclusion/Exclusion Criteria**

A small set of organisms should impact inclusion and exclusion criteria for clinical trials evaluating the impact of immunosuppression on type 1 diabetes. Organisms in this set include human immunodeficiency virus (HIV), *Mycobacterium tuberculosis*, hepatitis B virus (HBV), and hepatitis C virus (HCV). Recommended screening tests are available for these pathogens, but these tests have a window period (WP) of weeks to months from the time of infection to detection by a screening assay. The Mantoux tuberculin skin (purified protein derivative [PPD]) or interferon (IFN)-γ ELISPOT testing (IFN-γ release assay [IGRA]) is recommended for screening for *M. tuberculosis*; both have WPs of approximately 3 months. Purified protein derivative should be used to screen children <5 years old because IGRA testing is currently not recommended in younger children [12]. In certain circumstances, interpretation of a positive PPD, but not the IGRA, may be confused by prior receipt of Bacillus Calmette-Guérin vaccination, which can result in a false-positive skin test. Serologic testing is recommended for HIV (WP of 3–6 weeks, which should be shorter with the newer combined antigen/antibody tests) and HCV. Hepatitis B virus screening should be accomplished through a combination of hepatitis B core antibody (HBCAb) (WP of 4 months) and hepatitis B surface antigen (HBsAg) (WP of 2–4 weeks). It is recommended that these tests should be undertaken in all potential research participants, and a positive result for 1 or more of these pathogens should be considered as potential exclusion criteria. It is important to note that potential subjects with “old resolved” HBV (HBCAb+ and HBsAg–/HBV viral load–) may serorevert and develop active HBV infection with hepatitis [13]. The potential impact of new therapies to successfully treat HBV and HCV or secondary prophylaxis for TB is uncertain at this time in this setting. Individuals who have evidence or significant risk factors for HIV, HBV, or HCV may warrant further testing by nucleic acid test (NAT) in addition to serologic assessment.

**Potentially Important Pathogens That May Be Minimized by Monitoring**

The second category of pathogens includes those that are potentially important but whose impact may be minimized by monitoring. In general, these are viruses associated with life-long latent infection that frequently reactivate in the immunosuppressed host, potentially causing significant and more severe clinical disease in this population. The ability to follow serial viral load measurements in such patients may offer the opportunity to intervene preemptively in patients experiencing primary or reactivation infection. Such interventions may include temporary or permanent removal from study drug and, where appropriate, use of antiviral agents.

Important pathogens in this category are cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Cytomegalovirus and EBV are common opportunistic viral pathogens in immunocompromised hosts. Although CMV seroprevalence is low in infants in the United States, it increases throughout childhood and young adulthood (with community-acquired exposure such as in daycare settings), ultimately leading to rates ranging from 40% to 60% in healthy adults. The epidemiology of CMV varies by geographic region and socioeconomic status. Although infection of immunocompetent hosts typically results in asymptomatic or mildly symptomatic clinical presentations, CMV can cause significant morbidity and mortality in chronically immunocompromised hosts, including those on immunosuppressive therapy for autoimmune disorders [14–16]. Intensive immunosuppressive therapy, particularly those that target and result in depletion of T lymphocytes, results in the greatest risk for CMV reactivation and disease [15]. As seen in SOT patients [17], this risk is highest in patients who experience primary CMV infection that typically occurs in early childhood and adolescence. Clinical trials that target these age groups must consider both the risk of reactivation and the potential for acquisition of primary CMV infection in patients participating in the study.

Epstein-Barr virus is a ubiquitous herpesvirus that frequently infects children and adults. Rates of acquisition of EBV in children vary according to socioeconomic status with rates as high as 90% by 8 years of age seen in developing countries and lower socioeconomic populations in industrialized nations, compared with seronegativity rates of 30%–75% in adolescents in higher socioeconomic groups [18]. In contrast, up to 90% of healthy adults will
have serologic evidence of prior EBV infection. Although EBV acute infection is generally self-limited in immunocompetent individuals, development of EBV disease may be associated with significant morbidity and mortality in immunosuppressed patients. The most important complication of this pathogen is EBV-associated lymphoproliferative disorder (LPD), which has been most frequently reported in SOT [19] and umbilical cord blood HSCT recipients [20]. Epstein-Barr virus-associated LPD encompasses a spectrum of pathologic diagnoses, ranging from B cell hyperplasia to lymphoma. Important risk factors for the development of EBV disease in immunosuppressed patients include primary EBV infection during the time of immunosuppression, young age, and intensity of immunosuppression. In contrast to CMV, immunosuppressive agents that deplete or impair the function of T lymphocytes are most likely to be associated with increased risk of developing EBV disease and LPD [19]. Of note, the evaluation of anti-CD3 monoclonal antibody in new onset type 1 diabetes identified that 75% of patients receiving this agent experienced a syndrome similar to mononucleosis; 21 of 22 subjects receiving this therapy experienced transient increases in EBV load in the peripheral blood, although there was no apparent clinical sequelae [22]. Accordingly, the potential impact of EBV infection is of concern for individuals with new onset diabetes who are enrolled in trials and use immunosuppressive agents.

Because of the potential importance of these pathogens, we have developed specific strategies for monitoring CMV and EBV. In general, we consider the likely impact of immunosuppression regimens to be evaluated in each proposed trial because we consider the potential risk for CMV and EBV disease with these regimens. For subjects being evaluated for enrollment into protocols using significant immunosuppressive therapy, there should be consideration for assessment of past infection (and current infection in younger individuals) with CMV and EBV at baseline to determine the risk of reactivation and primary infection. We recommend obtaining serologies for both viruses during pre-enrollment screening and measurement of viral loads in those that are seronegative. Blood samples are routinely stored at various time points during a clinical trial and may be used for viral load measurements by NAT as directed by clinical illness. For those subjects found to have active primary infection during pre-enrollment screening, we recommend that the subject be excluded until the acute infection has resolved. For protocols that involve highly immunosuppressive agents with a demonstrated or presumed risk of CMV or EBV disease, real-time testing of CMV and EBV in seropositive subjects can be performed by viral load measurement in subjects with clinical symptoms compatible with these pathogens. Whenever feasible, sequential surveillance specimens could be obtained for retrospective NAT to help define the natural history of viral reactivation with study treatment [23]. Throughout the course of the study, careful evaluation of primary infection should be undertaken if a compatible syndrome develops. Because we do not fully understand the risk of infection or reactivation in this patient population, subjects involved in less intensive protocols can have specimens collected for testing retrospectively. Specimens should be collected and viral load reported in real-time in any subjects who develop symptoms consistent with viral disease, regardless of the level of intensity of the immunosuppressive regimen.

Identification of elevated viral loads for CMV and EBV potentially requires action by investigators. First, an assessment must be made regarding the clinical relevance of positive results particularly in seropositive individuals, as discussed below. Decisions must then be made regarding the need to temporarily or permanently discontinue study medications as well as to consider the potential use of therapeutic antiviral agents. It should be noted that discontinuation of the immunosuppressive agent may not result in immediate elimination of its immunosuppressive effect, which may significantly outlast the actual dosing period. We recommend that an algorithm defining the response to positive results be determined before trial initiation to assure a consistent approach study wide, but that infectious disease experts should be involved in the management.

The use of viral load monitoring using NAT is associated with several caveats that must be considered. One of the most important of these is the fact that because of the intra-laboratory variability of NAT results, a central laboratory should be used, particularly when monitoring trends. A second caveat is the fact that we do not fully understand the meaning of a low-level positive viral load (particularly for EBV) in seropositive populations because this has not been studied well in the normal population (and may not be uncommon) [24, 25] or in patients with type 1 diabetes. Further research is needed to elucidate the significance of low-level viremia in otherwise asymptomatic individuals.

Other Pathogens Commonly Seen in Immunosuppressed Individuals

There are a large number of other pathogens with the potential to cause disease in immunocompromised hosts.
The severity of disease is often dependent on the degree of immune suppression as well as the component of the immune system affected by the immunosuppressive agent(s). Although a history of these infections does not exclude participation in immunosuppressive trials, extra precautions and monitoring may be warranted. Examples of these are described in the following sections.

**Other Herpesviruses.** Other herpesviruses includes varicella zoster virus (VZV), herpes simplex virus (HSV) 1 and 2, and human herpesvirus (HHV)-6 A and B, HHV-7, and HHV-8. Like CMV and EBV, these viruses are often acquired in childhood and are associated with lifelong infection, raising the issue of both risk of primary acquisition as well as reactivation in the presence of immune suppression. Many of these viruses demonstrate a high prevalence of infection among adolescents.

The disease profiles of VZV and HSV are well described. These viruses are latent in the dorsal root ganglia and may periodically reactivate. The propensity to cause reactivation disease typically increases with immune suppression. Acquisition of primary VZV infection (chickenpox) may be more concerning than reactivation disease (herpes zoster or shingles) and would be more likely in unvaccinated pediatric subjects in temperate climate countries (tropical countries have sizable adult populations susceptible to VZV). Development of primary VZV infection would likely require discontinuing immunosuppressive medication and initiating antiviral therapy. Documentation of chickenpox, or varicella vaccination or VZV antibody measurement, should be considered during the study screening process. If there is a VZV exposure during the course of the study, these data may be useful in determining the risk for disease. For children without a proper VZV vaccination or infection history who are found to be VZV seronegative, immunization should be considered before study enrollment, although critical time frames for study participation might preclude this. If an exposure occurs, contact with an infectious diseases specialist should be considered to determine whether postexposure prophylaxis is warranted. A history of HSV infection should also be obtained. Patients with a history of frequent recurrence of oral or genital HSV infection might benefit from antiviral prophylaxis at the onset of the clinical trial, whereas infrequent reactivation may only require treatment of outbreaks. Development of HSV (primary or reactivation) during the trial may lead to systemic disease, require antiviral therapy, and may require holding study medication.

**Respiratory Viruses.** Respiratory viruses, including adenovirus, influenza, parainfluenza, RSV, and metapneumovirus, are ubiquitous and the likelihood of exposure during participation in a clinical trial is high. Although some of these viruses occur seasonally, others may occur at any time of year. Manifestations of infection can be variable, but patients receiving immunosuppressive medications are at increased risk for more severe disease; younger children may be at further increased risk for adverse outcomes. For this reason, potential subjects should be immunized with inactivated influenza vaccine (when seasonally available) at least 2 weeks before enrollment, if possible, or as soon as available if on therapy. In addition, patients who have symptoms that are potentially consistent with an active respiratory tract infection should have trial enrollment delayed until the illness is resolved; if the patient is already enrolled in the study, medication should be held until symptoms have fully abated. Patients and household contacts should be clearly instructed regarding measures to diminish the spread of respiratory viruses, including hand hygiene and immunization of household contacts with inactivated influenza vaccine. During influenza season, chemophrophylaxis with neuraminidase inhibitors (oseltamivir or zanamivir) may be considered for individuals with a significant exposure or risk of exposure who could not receive influenza vaccine [26]. Currently, there is no recommendation for use of antibody therapy with either intravenous immune globulin or RSV monoclonal antibodies in this patient population. Other than optimizing hand and respiratory hygiene within the home, there are no approved interventions available for adenovirus, parainfluenza, and metapneumovirus.

Symptoms of respiratory viral infections may be variable but are more likely to be severe in patients receiving immunosuppressive medications. Patients with upper or lower respiratory symptoms, myalgias, and undiagnosed febrile illnesses should be counseled to seek medical attention. Optimally, nasopharyngeal specimens should be collected for detection of respiratory viruses using molecular detection assays or antigen detection during the symptomatic period.

In most cases of respiratory viral infection, treatment is supportive. Antiviral therapy is only clearly established for influenza virus. For individuals suspected of having influenza, prompt initiation (within 48 hours) of a neuraminidase inhibitor (oseltamivir or zanamivir for influenza A or B strains) should be considered. The role of antiviral therapy for other respiratory viruses remains controversial. Although a number of new antiviral agents are undergoing evaluation for treatment of respiratory viruses, results of these trials are currently not available to inform clinical decisions.

**Pneumocystis jirovecii.** There are no specific guidelines for preemptive screening for pneumocystis infection in patients who are receiving immunosuppressive therapies for treatment of chronic conditions. If patients will be
receiving prolonged immunosuppression that is anticipated to cause prolonged CD4 count depression (<200/mm³) or have a significant use of glucocorticoid therapy (eg, >20 mg/day for more than 30 days), preventive therapy with either trimethoprim-sulfamethoxazole, dapsone, or atovaquone should be considered [27]. There is no standard screening procedure for asymptomatic patients; however, individuals with respiratory symptoms should be assessed for active infection with examination of sputum or bronchoscopically obtained respiratory specimens.

**Rare But Important Opportunistic Infections of Potential Clinical Significance**

There are a number of infections that, although rare even in immunocompromised hosts, can have significant clinical consequences and need to be considered in the appropriate clinical context. For example, unexplained neurologic findings may be due to progressive multifocal leukencephalopathy (PML) caused by John Cunningham virus or tropical spastic paraparesis caused by human T-lymphotropic virus (HTLV)-1. The recent finding of PML in patients receiving anti-CD20 monoclonal antibody therapy and cytotoxic T lymphocyte antigen-4 immunoglobulin requires increased vigilance regarding these serious complications with other types of monoclonal therapy [28]. For these and other rare infections, it is important to not only look at your own trial but also to look across trials or postlicensure use (including use in other patient populations) to identify potential signals of concern. Although reported in SOT recipients, HTLV-1-associated infections have not been described yet in patients with autoimmune disorders in association with specific immunosuppressive treatments, possibly due to the epidemiology of this infection [29]. Certain geographically restricted pathogens such as Coccidioides immitis/posoda and Histoplasma capsulatum may cause significant illness in patients undergoing immunosuppression and should be considered in patients who may have been exposed [30, 31]. Nevertheless, it is important to recognize the potential for these infections in patients undergoing treatment with immunosuppressive agents, and systematic assessment depending on the clinical syndrome should be performed such as with neurological changes (computed tomography or magnetic resonance imaging, lumbar puncture, and NAT) as appropriate. See the online supplement for further discussion of potential pathogens and an approach to monitoring.

**CONCLUSIONS**

There has been a significant expansion of the number and variety of immune-modulating agents over the past decade. These agents have the potential to significantly improve and potentially cure a large number of autoimmune and rheumatologic disorders, possibly including type 1 diabetes. The Type 1 Diabetes TrialNet Group has approached infection risk through a variety of mechanisms using the expertise of a group of infectious diseases and immunology specialists with specific expertise in infections in immunocompromised individuals in both study design and implementation. As such, this structure provides an approach to minimize infectious risks associated with the use of novel potent immunosuppressive therapies in research.

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**References**


Appendix (online)
A. Additional Pathogens and Pathogens of Lesser Concern, Vaccine and Travel Issues and Implementation
B. Approach to TrialNet Subjects with a Positive CMV PCR Viral Load
C. Approach to TrialNet Subjects with a Positive EBV PCR Viral Load
D. Type 1 Diabetes TrialNet Study Group