Update on viral infections in immunocompromised patients

Rafael de la Cámara[a], Javier López-Jiménez[b], Carlos Vallejo[c], Lourdes Vázquez[d], José Luis Pérez[e], Evaristo Varo[f], Luis Madero[g], José Mensa[h] and the Multidisciplinary Network of Experts[i]

[a]Hospital Universitario de la Princesa. Madrid. Spain. [b]Hospital Ramón y Cajal. Madrid. Spain. [c]Clínica Puerta de Hierro. Madrid. Spain. [d]Hospital Clínico. Salamanca. Spain. [e]Hospital Son Dureta. Palma de Mallorca. Spain. [f]Hospital Clínico Universitario. Santiago de Compostela. La Coruña. Spain. [g]Luis Madero. Hospital Niño Jesús. Madrid. Spain. [h]José Mensa. Hospital Clínic. Barcelona. Spain. [i]The remaining members of the network of experts were: Dr. Javier Garau, Mutua de Terrassa. Terrassa. Barcelona. Spain. Dr. Pilar Luque, Hospital Clínico. Zaragoza. Spain. Dr. Albert Pahissa, Hospital Vall d'Hebrón. Barcelona. Spain. Dr. Joaquín Portilla. Spain. Hospital General. Alicante. Dr. Miguel Salavert, Dr. Miguel Ángel Sanz and Dr. Amparo Solé, Hospital La Fe. Valencia. Spain.

Viral infections are one of the most frequent complications of oncohematologic and transplanted patients. Here we review some of the most interesting clinical publications of 2004 and 2005. A brief introduction of each virus or issue is given followed by a discussion of the related publications.

In relation to CMV we reviewed: 1) the recommendations of the European Group for Blood and Marrow Transplantation for CMV management in stem-cell hematopoietic transplants (HSCT); 2) a meta-analysis that evaluated the efficacy of different strategies for the prevention of CMV in solid organ transplants; 3) the impact of CMV disease before HSCT in the outcome of the transplants.

A recent publication found a relation between herpesvirus 6 infection and increased mortality. This is a novel finding, not previously described, that reinforces the importance of the so-called "indirect effects" of the beta-herpesvirus.

In the largest study performed until now of influenza in HSCT recipients, early antiviral therapy with oseltamivir was more effective than rimantadine for both preventing progression from influenza-A upper respiratory infection to pneumonia and reducing influenza-A pneumonia mortality.

The human metapneumovirus (hMPV) is a newly described virus identified in 2001. The epidemiological and clinical characteristics of hMPV respiratory infections were described in a large group of oncohematologic patients.

A novel approach was published for the treatment of HLTV-1 infections based on the pathogenesis of this infection.

Finally, an exciting paper from the Perugia group showed that cloning of pathogen-specific T-cells seems a promising approach in the management of certain infections after HSCT.

Key words: **Viral infections. Immunocompromised patients. Stem-cell hematopoietic transplants. Solid organ transplants. Herpesvirus. Adoptive specific cellular immunotherapy. CMV. Herpes simplex virus. Human T-cell leukaemia virus type I. Community-acquired respiratory viruses. Influenza. Human metapneumovirus.**

Actualización de las infecciones víricas en los pacientes con inmunosupresión

Las infecciones víricas constituyen una de las complicaciones más frecuentes de los pacientes oncohematológicos y de los que reciben un trasplante. En este documento se revisan algunas de las publicaciones clínicas más interesantes de 2004 y 2005. Se ofrece una breve introducción de cada virus o proceso patológico, seguida de la discusión de las publicaciones relacionadas.

En lo relativo al citomegalovirus (CMV), se han revisado: *1)* las recomendaciones del European Group for Blood and Marrow Transplantation respecto al tratamiento del CMV en los trasplantes de células progenitoras hematopoyéticas (TPH); *2)* un metaanálisis en el que se evaluó la eficacia de las distintas estrategias para la prevención de la infección por CMV en los pacientes intervenidos mediante trasplante de órganos sólidos; *3)* la repercusión de la infección por CMV antes del TPH en lo relativo a la supervivencia del trasplante.

En una publicación reciente se demuestra la existencia de una relación entre la infección por el virus herpes 6 y el incremento de la mortalidad. Este hallazgo es novedoso, en el sentido de que no se había descrito previamente, y refuerza la importancia de los denominados «efectos indirectos» de los virus herpes-beta.

En el estudio más amplio efectuado hasta el momento del virus influenza en receptores de TPH, el tratamiento antivírico temprano con oseltamivir fue más efectivo que la administración de rimantadina tanto para la prevención de la progresión de la infección del tracto respiratorio superior por el virus de la gripe A hacia neumonía como en la reducción de la mortalidad por neumonía.

Correspondence: Dr. R. de la Cámara.
Hospital Universitario de la Princesa. Hematology Department.
Diego de León, 62. 28006 Madrid. Spain.
E-mail: jrcamara@telefonica.net

El metaneumovirus humano (MNVh) fue descrito en 2001. Las características epidemiológicas y clínicas de las infecciones respiratorias causadas por MNVh fueron evaluadas en un grupo grande de pacientes oncohematológicos. Se ha propuesto un nuevo enfoque en el tratamiento de las infecciones causadas por el virus de la leucemia T humana del adulto tipo I, fundamentado en la patogenia de esta infección. Finalmente, en un artículo muy interesante del grupo de Perugia se demuestra que la clonación de linfocitos T con especificidad de patógeno parece constituir un abordaje prometedor en el tratamiento de ciertas infecciones asociadas al TCPH.

Palabras clave: **Infecciones víricas. Pacientes con inmunosupresión. Trasplantes hematopoyéticos de células progenitoras. Trasplantes de órganos sólidos. Virus herpes. Inmunoterapia celular específica adoptiva. CMV. Virus herpes simple. Virus de la leucemia T humana tipo I. Virus respiratorios adquiridos en el medio extrahospitalario. Gripe. Metaneumovirus humano.**

State of the art (Dr. R de la Cámara)

Infection, particularly viral infection, is the most frequent complication of oncohematologic and transplant patients. The herpesvirus group causes most clinically important infections, although other viruses, such as community-acquired respiratory viruses (CRVs), are increasingly recognised as a significant cause of morbidity and mortality in this population. Here, we review some of the most interesting publications of 2004 and 2005 according to a multidisciplinary group devoted to the care of immunocompromised patients. As the topic is very broad, we have focused on clinical publications related to herpesvirus, community-acquired respiratory viruses, human T-cell leukemia virus type I (HTLV-1), and adoptive specific cellular immunotherapy[1-9]. A brief introduction to each type of virus will be given below, followed by a discussion of the publication.

Viruses affect the clinical outcome of immunocompromised patients in two different ways: by the direct effects of the virus, in the form of a recognized viral disease, for example CMV pneumonitis; and by so-called "indirect effects", which are increasingly recognised as an important part of the whole viral effect. These consist of clinical events associated with virus seropositivity or the development of viral infection but not with the viral disease itself. These effects have been shown in solid organ (SOT) and hematopoietic stem-cell transplants (HSCT)[10-12] and include graft rejection, increased incidence of graft-versus-host disease (GVHD), bacterial and fungal infections, transplant-related mortality, and decreased survival[13-17]. The paradigm of these direct-indirect viral effects is CMV, although other viruses, such as other beta-herpesvirus and CRVs are emerging as examples of the two effects of viral pathology[4,6,18,19]. The indirect effects may produce greater morbidity and mortality than the direct effects of the virus; therefore, the prevention of viral infection has broader implications than the prevention of the direct short-term consequences of viral diseases. Knowledge of the risk factors for the different viral infections and the development of guidelines for their management are of vital clinical importance. Below we summarize various publications related to these issues[1,3,4,6].

Community-acquired respiratory viruses (CRVS) are increasingly recognized as a significant cause of morbidity and mortality in oncohematologic and transplanted patients[19]. Approximately 1% of allogeneic-HSCT recipients die of CRV infection[20], which is not too different from current CMV-attributed mortality. In a recent large prospective multicenter study, CMV-associated mortality was 2.2%[21]. This study[20] and many others, probably underestimate the true incidence of CRV infections and attributed mortality in this population, one reason being that testing has used culture-based techniques or antigen detection tests that lack sensitivity for the diagnosis of respiratory viral infections. Highly sensitive real-time quantitative reverse transcriptase PCR (RT-PCR) assays can detect even more cases of CRV infection than previously reported. These techniques have doubled or tripled the detection rate of CRVs among immunocompromised adults[19]. Moreover, as shown previously, CRV infections are associated with several indirect effects, such as increased long-term mortality after HSCT—even after upper respiratory tract infections[18]—and increased incidence of invasive aspergillosis[16]. As with CMV, mortality related to CRV infections is not only mediated via viral pneumonitis and acute pulmonary failure. Moreover, new viruses are continuously being discovered. A recent example is the metapneumovirus. The implication of these new viruses in the immunocompromised host is an area that needs continuous clinical research[5].

Antiviral resistance in herpesvirus is an increasing concern in the transplant setting. In HIV-infected patients, drug-resistant CMV has not been a frecuent problem since the introduction of highly active antiretroviral combination therapy (HAART). CMV and herpes simplex are the most common resistant viruses in clinical practice[22,23], and large-scale studies that assess their development over time are welcome[7].

Viruses frequently cause opportunistic infections in oncohematology patients. However, the role of viruses as the cause of hematological malignancies is another interesting aspect of viral pathology. The case of human T-cell leukemia virus type I (HTLV-1) with adult T-cell leukemia (ATL) is a good example. The advance in knowledge of the molecular pathways of viral infections can result in new forms of therapy for diseases that, until now, had an ominous prognosis, e.g., ATL. The study by Watanabe et al is a brilliant example of this possibility[8].

The use of adoptive specific cellular immunotherapy is an interesting alternative approach for the prevention and treatment of viral infections. In HLA identical HSCT, successful results have been published for CMV[24] and EBV[25]. HLA barriers have been considered a major obstacle for the spread of these therapies to the mismatch HSCT. The recent publication by the Perugia group[9] challenges this view, showing that an effective and safe therapy is possible even in the case of maximum HLA mismatch (haploidentical transplant).

Herpesvirus infections

CMV

Among oncohematologic and transplanted patients, those who receive an allogeneic-HSCT are the subject of most studies on viral infections. Undoubtedly, CMV is the most representative of the opportunistic viral infections and for many years was the cause of most virus-related deaths. Mortality due to CMV was alarming: approximately between 1 out 5 and 1 out 10 patients died due to CMV disease[26]. Improvements in CMV disease therapy have been very modest during the last 15 years, but the prevention of CMV disease with different strategies has been one of the major advances in HSCT, with a huge impact on the global survival of the patients. Therefore, a good prevention policy for CMV is a basic requisite of all HSCT programs. The recommendations of the Infectious Diseases Working Party (IDWP) of the European Group for Blood and Marrow Transplantation (EBMT) provide a welcome evidence-based synthesis of the state of the art for CMV prevention.

Ljungman P, Reusser P, De la Camara R, Einsele H, Engelhard D, Ribaud P et al. Management of CMV infections: recommendations from the Infectious Diseases Working Party of the EBMT. Bone Marrow Transplant. 2004;33:1075-81.

These are the recommendations of the IDWP of the EBMT, graded according to the guidelines of the United States Centers for Disease Control (CDC). The recommendations are divided into different sections: pre-transplant management and donor selection, diagnosis of CMV infection and disease, and prevention and treatment of CMV disease. A summary of the main recommendations is presented here.

In relation to donors, there is a clear recommendation for a CMV seronegative donor if the patient is found to be seronegative (AI). If the patient is found to be seropositive, the choice of the proper donor based on CMV serology is controversial. All allogeneic-HSCT patients, regardless of whether or not they receive CMV prophylaxis, should be monitored after transplant for CMV with peripheral blood sampling at least weekly, using either the CMV antigenemia assay or a technique for the detection of either CMV DNA or RNA (AI), for at least 100 days (AI). Longer monitoring is recommended in patients with acute or chronic GVHD, those who experienced an earlier CMV reactivation and those who underwent mismatched or unrelated donor transplantation (BII). As a general rule, it is not recommended that autologous-HSCT patients be routinely monitored for CMV, although certain high-risk patients might potentially benefit from monitoring and the use of pre-emptive therapy (BII).

Accepted CMV disease definitions should be used. These definitions have been published in detail elsewhere (10).

These recommendations are aimed mainly at preventing CMV disease. The exclusive use of leukocyte-depleted or CMV-seronegative blood products is strongly recommended for CMV seronegative allogeneic-HSCT patients with CMV seronegative donors (AI) and merely recommended for CMV seronegative autologous-HSCT patients (BII). It should be pointed out that immunoglobulin for the prevention of CMV infection or disease is not recommended (DII), despite its broad use in practice. These recommendations consider the strategy of pre-emptive antiviral therapy based on the detection of CMV antigen or nucleic acid as the first-line preventive strategy for allogeneic-HSCT patients (AI). Intravenous ganciclovir prophylaxis is an effective strategy for the prevention of CMV disease and could be used in subgroups of allogeneic-HSCT patients, perceived to have a high risk for CMV disease (AI). If a low potency anti-CMV drug, such as acyclovir or valacyclovir, is used as prophylaxis in allogeneic-HSCT it must be combined with monitoring and pre-emptive therapy (AI). CMV disease, in particular CMV pneumonia, is still associated with a high mortality and should be regarded as a failure of the preventive strategy. Today's experience comes from uncontrolled therapeutic studies. A combination of intravenous ganciclovir and immune globulin is recommended for the therapy of CMV pneumonia (BII). For other types of CMV disease, either intravenous ganciclovir or foscarnet administered without immunoglobulin is recommended (BII). Cidofovir or the combination of intravenous ganciclovir and foscarnet can be used as second-line therapy for CMV disease (BII).

Fries BC, Riddell SR, Kim HW, Corey L, Dahlgren C, Woolfrey A et al. Cytomegalovirus disease before hematopoietic cell transplantation as a risk for complications after transplantation. Biol Blood Marrow Transplant. 2005;11:136-48.

For many years, CMV disease was considered of interest only for "transplant physicians", as its incidence in non-transplant patients was very low[27]. However, nowadays, CMV disease is emerging in several populations of non-transplant patients[28,29]. As a consequence, CMV disease in candidates for HSCT is increasingly common. Nonetheless, the effect of a history of CMV disease on outcome after HSCT is not known. The experience of the Fred Hutchinson Cancer Research Center (FHCRC) discussed here is an important step toward understanding the consequences of this problem. This group retrospectively analyzed 22 patients with CMV disease referred for HSCT between 1986 and 2000. The purpose of the study was to review the clinical presentation, course and response to treatment of these pre-transplant CMV diseases, and to determine the outcome of the patients who finally proceeded to HSCT. Pre-HSCT CMV disease is a rare event, although it has tragic consequences for the patients that finally receive an HSCT. During the study period, 4187 patients underwent allogeneic-HSCT and 1250 underwent autologous-HSCT at the FHCRC. The estimated incidence of CMV disease before HSCT in allogeneic transplant recipients was 0.28% and for autologous transplant recipients it was 0% (no case was identified in candidates for autologous transplant). Most of the patients with pre-transplant CMV disease presented with pneumonia and half were simultaneously infected with other pathogens. Half of the patients presented with mild clinical signs, including a normal chest radiograph, or atypically making the diagnosis of the CMV disease difficult. A high degree of suspicion is necessary to prompt appropriate diagnos-

tic studies and to administer the appropriate treatment. Nonetheless, the mortality directly attributed to CMV was relatively low (2/22). Only 14 out of 22 patients with CMV disease eventually underwent HSCT. All, except one case, received a myeloablative-conditioning regimen. The result was disastrous, with only one long-term survivor. These poor results were due to a very high incidence of CMV disease and high transplant-related mortality (TRM). Six patients developed CMV disease at a median of 42 days after transplantation, in spite of prophylaxis with high-dose acyclovir and ganciclovir on engraftment, or standard post-engraftment pre-emptive therapy strategies. Half of the CMV diseases were observed before day 20 post-transplant, which is very unusual.

Thus, the message of this report is clear. All patients with a history of CMV disease pre-transplant must be considered very high-risk cases for myeloablative allogeneic-HSCT. The conventional strategies for CMV disease prevention do not work properly in this context and more aggressive management is needed. New approaches should be planned. These include anti-CMV maintenance therapy until the start of the conditioning regimen, CMV-specific T-cell therapy, foscarnet prophylaxis until engraftment, and intensive CMV monitoring with highly sensitive assays (for example PCR with a threshold of 100 copies per millilitre).

Kalil AC, Levitsky J, Lyden E, Stoner J, Freifeld AG. Meta-analysis: the efficacy of strategies to prevent organ disease by cytomegalovirus in solid organ transplant recipients. Ann Intern Med. 2005;143: 870-80.

CMV is the most common opportunistic viral infection occurring after solid organ transplantation (SOT). It causes substantial morbidity, prolongs hospital stay, and increases healthcare costs and the risk of death. It is also associated with many adverse indirect effects, such as bacterial and fungal infections, graft rejection, atherosclerosis, and post-transplantation lymphoproliferative disorders. The prevention of CMV disease in SOT is therefore a basic part of all transplant programs. Pre-emptive treatment and universal prophylaxis are the two approaches used to prevent CMV disease in SOT patients. There is no agreement as to which is best in the prevention of CMV disease, or if either of the two is effective in preventing the indirect effects of CMV infection. The meta-analysis by Kalil et al summarizes 17 randomized trials that evaluated these strategies for the prevention of CMV and associated complications in liver and kidney recipients. The studies were published between 1989 and 2003, and involved 1980 patients. All the studies compared pre-emptive therapy or universal prophylaxis with placebo or no treatment. The meta-analysis had several exclusion criteria: uses of low doses of antivirals (< 2 g/day of acyclovir or < 3 g/day of ganciclovir), short periods of treatment (< 14 days) or prophylaxis (< 60 days), or active therapy to prevent CMV in the control group. The results of this meta-analysis are very interesting and are expressed as a percentage of reduction compared with placebo or no therapy. Both strategies were effective for reducing CMV organ disease (80% for prophylaxis and 72% for pre-emptive therapy) and in the rate of allograft rejection (26% for prophylaxis and 53% for pre-emptive therapy). However, in patients with the highest risk of CMV disease, only universal prophylaxis, and not the pre-emptive approach, reduced CMV organ disease and produced a statistically significant reduction in the incidence of bacterial and fungal infections (51%), non-CMV viral infections (84%) and death (38%). These data suggest that both approaches are effective in preventing CMV organ disease but that universal prophylaxis may provide additional benefits (less bacterial, fungal and non-CMV infections, and lower mortality). Another interesting finding of this meta-analysis was that high-dose acyclovir, used as universal prophylaxis, was effective in preventing CMV organ disease in the high-risk population (donor-positive/recipient-negative and recipient-positive serostatus). No direct comparison of acyclovir and ganciclovir was performed in the meta-analysis.

There are two main messages in this meta-analysis. First, universal prophylaxis may be the preferred strategy for CMV prevention in SOT patients. And second, the data analyzed support the concept that high-dose acyclovir should remain among the drugs of choice for universal prophylaxis in liver and kidney transplant recipients at high risk of CMV disease.

Human herpesvirus 6 (HHV-6)

HHV-6, a member of the β-herpes-virinae subfamily, was discovered in 1986[30]. It was etiologically linked to human disease in 1988 and first described in HSCT in 1991. It is further classified into two genetically distinct variants, A and B, which are serologically indistinguishable. CD46 is the receptor for this virus. It is a ubiquitous virus that infects almost all individuals in early childhood. Almost all children are HHV-6 seropositive by 2 years of age. HHV-6B has been conclusively proven to cause exanthema subitum (or sixth disease), but HHV-6A has not yet been firmly associated with any disease. Nonetheless, HHV-6A has been isolated from immunocompromised patients, and was considered the cause of death in a kidney transplant recipient[31], and in patients with neurological complications. It seems that HHV-6A may have a greater neurotropism than HHV-6B. Of all the herpesviruses, HHV-6 is unique due to its site-specific ability to integrate its genome into human chromosomes[30], with the result that a new virus transmission route, inherited from chromosomes of one or both parents, is possible, with an estimated prevalence of 0.2%[32]. HHV-6 has immunomodulating properties (30) and is associated with indirect effects in both SOT and HSCT[11,12].

In HSCT, HHV-6 infection is very common with 30-50% of patients developing viremia within the first few weeks after transplantation, usually before CMV reactivation. HHV-6 viremia is significantly higher among allogeneic-HSCT recipients than in autologous recipients. Asymptomatic HHV-6 reactivations predominate in the post-HSCT setting. Four major clinical events have been associated with HHV-6 infection in HSCT receptors: the development of fever and rash associated with HHV-6 viremia, encephalitis, bone marrow suppression, and pneumonitis. No impact on survival has been described to date[33].

Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical Outcomes of Human Herpesvirus 6 Reactivation after Hematopoietic Stem Cell Transplantation. Clin Infect Dis. 2005;40:932-40.

This article, the largest study performed to date, summarizes the FHCRC experience with HHV-6 in allogeneic-HSCT. The aim of the study was to evaluate the effects of HHV-6 reactivation on the clinical course of patients who have undergone allogeneic-HSCT. Quantitative PCR tests for HHV-6 were performed on prospective weekly plasma samples for 100 days after HSCT. A retrospective review of medical records determined clinical end-points. A total of 110 allogeneic-HSCT recipients were included. HHV-6 reactivated in 52 of the 110 patients at a median of 23 days after transplantation[19-28]. In the multivariate analysis, HHV-6 reactivation was associated with a lower probability of platelet engraftment (hazard ratio 0.47), an increased risk of subsequent acute GVHD grades III-IV (HR 4.9), and an increase in all-cause mortality at day 50 post-transplant (HR 2.9). Furthermore, a high level of HHV-6 viremia was associated with subsequent central nervous system dysfunction (HR 21). The relationship between HHV-6 infection and increased mortality is a novel finding, of this study not previously described. However, as the authors remark in the Discussion, whether this is a causal relationship or whether HHV-6 reactivation is merely a marker for other, unmeasured etiologies of mortality cannot be determined from this study. Nonetheless, the impact of HHV-6 viremia in subsequent acute GVHD and mortality reinforces the importance of the so-called "indirect effects" of the beta-herpesvirus.

Herpes simplex and antiviral resistance

Herpes simplex virus (HSV) is one of the most common infections worldwide and affects immunocompetent and immunocompromised patients. Acyclovir, launched in 1982, is still the standard treatment for HSV infections. Today, more than 20 years after its release, acyclovir continues to be one of the best-selling antiviral drugs worldwide. Previous surveys have shown a low prevalence of acyclovir-resistant HSV among immunocompetent patients (0-0.6%) and a significant prevalence among immunocompromised patients (3-6%). Acyclovir-resistant HSV can be isolated from treatment-naïve patients[34]. Acyclovir is increasingly used both in the immunocompetent and immunocompromised population. In the general population it is used mainly in self-medication for the treatment of labial herpes, and in immunocompromised patients, it is used prophylactically for long periods and also for repetitive curative treatments. Therefore, the scenario is favorable for an increase in acyclovir-resistant HSV. Is this occurring now? Danve-Szatanek et al[7] try to answer this worrying question.

Danve-Szatanek C, Aymard M, Thouvenot D, Morfin F, Agius G, Bertin I et al. Surveillance network for herpes simplex virus resistance to antiviral drugs: 3-year follow-up. J Clin Microbiol. 2004;42:242-9.

The aim of the study was to conduct a prospective and coordinated multicenter national study of the emergence of acyclovir-resistant HSV to establish a correlation between in vitro and clinical resistance, and to show possible cross-resistance among different antiviral drugs. For these purposes, a French surveillance network, formed by 15 virology laboratories, isolated and identified HSV-1 and HSV-2 strains among hospitalized subjects between May 1999 and April 2002. A total of 3923 strains corresponding to 3357 patients were included in the study. Fifty-five percent of the patients were immunocompetent and 45% immunocompromised. The main causes of immunosuppression were: blood disease (32%), HIV infection (21%), organ transplantation (13.5%) and HSCT (13.5%). Acyclovir-resistant HSV were excreted by 6 immunocompetent patients (0.3%) and by 54 immunocompromised patients (3.6%) ($p < 0.001$). The percentage of acyclovir-resistant HVS-2 strains was not significantly higher than the percentage of HSV-1 strains. One immunocompetent patient who had not previously received acyclovir was infected by an acyclovir-resistant strain. In immunocompromised patients the highest prevalence was seen not in HIV patients but in HSCT patients (10.9%). For the other groups of immunocompromised patients prevalence was 4.2% in HIV patients, 2.5% in SOT, 2.1% in blood diseases and 2.5% in the miscellaneous category. In HSCT, there was a significant difference between the prevalence in allogeneic patients (18.4%) and in autologous patients (1.2%) (p 0.001). The results of immunocompetent and immunocompromised patients were compared with the data of a previous survey from the early 1990s[35], and no increase over time was observed. Sixty-one percent of those who excreted acyclovir-resistant HSV and were treated with foscarnet developed resistance to this drug.

These data show that for immunocompetent patients, the prevalence of acyclovir-resistant HSV is very low (0.3%), not significantly different from the prevalence observed by other authors. For immunocompromised patients, the prevalence of resistance found (3.6%) was lower but not significantly different from that observed in other studies. Patients undergoing HSCT have the highest prevalence of resistance (10.9%), whereas among HIV-infected patients the prevalence is apparently declining. An interesting aspect of this study was the finding of a stable frequency of resistance over time both in the immunocompetent and immunocompromised populations.

In spite of the massive use of acyclovir, it seems that resistance to this agent has not increased during the course of the last 10 years. Foscarnet is the drug of choice for acyclovir-resistant HSV, but the high incidence of resistance to this drug found in this study is worrisome and has implications for patient management.

Community-acquired respiratory viruses (CRVS)

Influenza

The epidemiology of influenza in HSCT patients closely parallels the ocurrence of the infect on in the community, as do other CRVS, and can occurs as out breaks. Progression from upper respiratory infection (URI) to pneumonia occurs in approximately 20% of cases, thus making it a serious event since influenza pneumonia in HSCT has 20% mortality[19] (varying from 17% to 57%). This mortality

is lower than that caused by respiratory syncytial virus (RSV) and parainfluenza but, in contrast to these viruses, influenza has an effective vaccine and well-tolerated antivirals for its prevention and treatment. It should be pointed out that the European[36] and American guidelines[37] recommend annual immunization against influenza for family members of HSCT patients and staff of transplant units. Two types of antivirals are active against influenza: the newly licensed neuraminidase inhibitors (oseltamivir and zanamivir) and the old M2 inhibitors (amantadine and rimantadine).

Nichols WG, Guthrie KA, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis. 2004;39: 1300-6.

This article, which describes the largest study performed to date, summarizes the FHCRC experience with influenza infections occurring within the first 120 days after HSCT over 12 consecutive years (September 1989 through March 2002). A total of 4797 patients who underwent their first allogeneic, syngeneic, or autologous-HSCT were included. To prevent transmission, influenza vaccination was offered each fall to all healthcare workers and to family caregivers. Infected patients were placed in respiratory isolation until they were free of symptoms and nasopharyngeal secretions yielded negative results, and could receive antiviral treatment or not at the discretion of the attending physician. Antiviral treatment was an M2 inhibitor (rimantadine) or an oral neuraminidase inhibitor (oseltamivir), which has been available from 1999.

The prevalence of influenza infection was 1.3%. Risk factors for the acquisition of the infection were advanced hematological disease and female sex. Influenza pneumonia developed in 18 patients (29% of those infected), and it was closely related to influenza mortality (30-day mortality rate of pneumonia: 28%). Pulmonary copathogens (such as *Aspergillus fumigatus*, *Corynebacterium jeikeium*, or RSV) were commonly isolated in patients with influenza pneumonia. Severe lymphopenia (< 100/µl) at the time of diagnosis and treatment of URI with rimantadine or no antivirals was found to be an adverse risk factor for pneumonia. On the other hand, treatment of URI with oseltamivir and, surprisingly, the concomitant use of corticosteroids protected against pneumonia. In this series, early antiviral therapy with oseltamivir was more effective than rimantadine both for preventing progression from influenza-A URI to pneumonia (0% vs 13%), and for reducing influenza-A pneumonia mortality (0% vs 40%). Influenza shedding in nasopharyngeal secretions was shorter in patients on no or low doses of steroids (< 1 mg/kg) and in patients on oseltamivir therapy (trend). Influenza lower respiratory tract infection (LRI) was an independent risk factor for 1-year mortality (HR 2.6).

The influenza infection attack rate found in this study (1.3%) seems surprisingly low for the highly immunosuppressed population described. As the authors point out, this is probably due to the aggressive infection-control measures used at the FHCRC. These data support the recommendation to implement a vaccination program both for family caregivers and healthcare professionals, along with the screening and isolation of every symptomatic patient in all transplant centers. On the other hand, analysis of the data shows that influenza-related mortality is closely linked to the development of pneumonia, with or without the presence of copathogens. Consequently, preventing pneumonia among infected patients should be a main objective. Moreover, influenza mortality goes further than the short-term pneumonia mortality as shown by the independent effect of influenza LRI on 1-year mortality. Although the study presented here was not a randomized study and the number of treated patients was small, the data suggest that early antiviral therapy with oseltamivir is effective both for preventing progression of influenza-A URI and for treating influenza-A pneumonia. In addition, since oseltamivir seems to reduce viral shedding, it would prevent nosocomial transmission to others. The optimal duration of therapy has yet to be identified. These data extended the previous positive, although limited, experience with another neuraminidase inhibitor, zanamivir[38].

The effectiveness of neuraminidase inhibitors and the ineffectiveness of the M2 inhibitors in this study have practical implications for patient management and are particularly relevant in the present context of rapidly evolving influenza resistance to antivirals. Recent data showing an alarming increase in resistance to M2 inhibitors reinforce the use of the neuraminidase inhibitors. During the 2004–2005 influenza season, approximately 70 percent of the influenza-virus isolates from China and Hong Kong, and nearly 15 percent of those from the United States and Europe showed resistance to M2 inhibitors[39]. But new data are even more worrisome. According to the CDC, between October 1 and December 31, 2005, ninety-two percent of the influenza-A viruses isolated in the United States were resistant to adamantine derivatives (amantadine and rimantadine)[40]. This means that adamantine derivatives are no longer effective for prophylaxis or treatment in large areas of the world. On the basis of available antiviral testing results, the CDC recommended that neither amantadine nor rimantadine be used for the treatment or chemoprophylaxis of influenza A infections in the United States for the remainder of the 2005–06 influenza season[41]. During this period, oseltamivir or zanamivir were to be used for the treatment of influenza, or oseltamivir for chemoprophylaxis of influenza[41].

Human metapneumovirus (hMPV)

The human metapneumovirus (hMPV) is a newly described member of the *Paramyxoviridae* family belonging to the *Metapneumovirus* genus. hMPV was identified in 2001 from young children in The Netherlands, but is not a real new virus for humans[42,43]. We now know that it has been circulating at least since 1950, it has been found in most parts of the world, and infects almost all individuals by 5-10 years of age. It has been associated with acute upper and lower respiratory-tract infection in children and adults, and the diseases caused by hMPV are similar to those caused by RSV. hMPV infection is somewhat less common than infection with RSV but more common than parainfluenza virus infection. It is difficult to make such

comparisons with certainty since they depend on the relative sensitivity of the detection systems. Metapneumoviruses are diagnosed in clinical practice by PCR techniques that are probably more sensitive than other detection methods for most viruses. It is isolated from 10% (varying from 7 to 43%) of patients with acute respiratory tract infections without a known etiology. Its seasonality is similar to that of RSV. hMPV can cause severe infections in immunocompromised patients. In HSCT patients, the first fatal case was described in 2003[44].

Williams JV, Martino R, Rabella N, Otegui M, Parody R, Heck JM et al. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. J Infect Dis. 2005; 192:1061-5.

In this paper, the authors analyze 304 episodes of upper and lower respiratory infection (URI & LRI) occurring in 128 adult hospitalized-patients with hematologic malignancies at the Santa Creu i Sant Pau Hospital in Barcelona, Spain, over 5 consecutive years (October 1999 through July 2004). The epidemiological and clinical characteristics of hMPV respiratory infections are described and compared with their own series of other respiratory virus infections such as influenza virus, RSV, parainfluenza virus (PIV), adenovirus, rhinovirus and enterovirus.

Patients with symptoms of URI underwent nasopharyngeal aspiration, whereas patients with LRI underwent bronchoalveolar lavage when possible. All samples were tested using viral direct fluorescent antibody assays (DFA) for influenza (A and B), RSV, PIV (1, 2 and 3) and adenoviruses, cultured for viruses, and tested by reverse-transcription polymerase chain reaction (PCR) for enterovirus and hMPV (retrospectively, in frozen samples in the latter case).

Respiratory viruses were found in 51% of the episodes of respiratory infection, the most frequent being influenza virus in 22%, RSV in 9%, hMPV in 9%, parainfluenza virus in 5%, and adenovirus in 4%. More than 1 virus was found in 17% of the episodes. Of particular interest is the fact that 41% of patients with hMPV were coinfected with other viruses. Among the hMPV-infected patients, 73% were HSCT recipients (mean: day +144; range: day + 1 - day + 488). A significant proportion of hMPV infections occurred during spring (41%) and summer (18%), unlike influenza (13% and 4%, respectively) and RSV (18% and 0%, respectively). Although most hMPV-infected patients presented with URI alone, 41% developed LRI (comparable to the rates of patients with RSV and influenza). Risk factors for progression from URI to LRI included lymphopenia < 200/µl (HR 7.8), infection with hMPV or RSV versus other viruses (HR 4), and having received an allogeneic-HSCT (HR 3.2). hMPV LRI-related mortality was 33%; in 2/3 cases, bacterial copathogens were a potential cause of death.

As the authors point out, comparisons between the prevalence of hMPV and other viruses in this cohort are difficult to make since hMPV was detected by PCR, a much more sensitive technique than those used to detect most of the other viruses (DFA, viral culture). A further limitation of the study is the heterogeneity of underlying diagnoses and the degree of immunosuppression. However, the prospective nature of the study and the recruitment of patients over a 4-year period provide a good framework to define the epidemiological and clinical characteristics of respiratory viruses and, particularly, of hMPV infection in immunocompromised adults.

Respiratory viruses were found in around half of the episodes of respiratory infection, which reinforces the importance of these agents in the etiology of respiratory infections of hematologic patients. In the HSCT setting, during the study period, 23% of the HSCT recipients had a respiratory virus infection at any given time. hMPV was isolated in 9% of all the episodes of respiratory infection and in 17% of pneumonias. Most of the hMPV-infected patients were HSCT recipients. hMPV infections occurred in any season, summer included. Even though a considerable proportion (41%) of hMPV infected patients were coinfected with other viruses, hMPV was the primary virus isolated in the vast majority of cases, which suggests that it alone was responsible for the associated respiratory illness. hMPV-related mortality was closely linked to the development of LRI, with or without copathogens, as happens with the remaining respiratory viruses.

These data suggest that hMPV is a significant respiratory pathogen in the population studied and that it should be actively sought in immunocompromised patients with upper or lower respiratory disease, particularly in the HSCT setting, where it occurs throughout the year.

Human T-cell leukemia virus tipe I (HTLV-1)

HTLV-1, the first-described human retrovirus associated with disease, is the causative agent of adult T-cell leukemia/lymphoma (ATLL) and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM)[45]. An estimated 10 to 20 million people worldwide are infected with HTLV-1 virus. The infection is endemic in the Caribbean, parts of Africa, southwestern Japan, and Italy. Most of the infected individuals remain lifetime asymptomatic carriers, but between 1 and 4% develop ATLL. In some countries, ATLL is a serious practical problem. About 1000 new cases are diagnosed each year in Japan alone. Chemotherapy, monoclonal antibodies, recombinant immunotoxins and bone marrow transplant have been used to treat ATLL, with little success. In fact, mean survival after ATLL diagnosis is about one year with only a few patients achieving sustained long-lasting remissions. The impact of an effective treatment/chemoprophylactic strategy in this virus-related blood disease would be enormous.

ATLL leukemogenesis is a multistage process in which a virus-encoded protein, Tax (transcriptional activator), seems to play a critical role. Tax has been identified as the transforming protein of HLTV-1. At first, Tax induces chronic polyclonal T-cell proliferation, dysregulating the growth of infected cells and immortalizing them (at least *in vitro*). Later, Tax may facilitate the progression of infected T-cells to a transformed phenotype by a) impairing the cell's ability to repair DNA damage, b) functionally inactivating p53 to allow proliferation and survival in

spite of genomic damage, and c) activating nuclear factor-kappa beta (NF-κβ) that protects ATLL cells from apoptosis and contributes to aberrant growth and cytokine gene expression. Constitutive activation of NF-κβ in the leukemic cell is essential for their growth and survival. In this process, genetic defects accumulate in T-cells, with the corresponding progression to a transformed leukemic phenotype.

Watanabe M, Ohsugi T, Shoda M, Ishida T, Aizawa S, Maruyama-Nagai M et al. Dual targeting of transformed and untransformed HTLV-1-infected T cells by DHMEQ, a potent and selective inhibitor of NF-kappaB, as a strategy for chemoprevention and therapy of adult T-cell leukemia. Blood. 2005;106: 2462-71.

Using the pathogenesis of HTLV-1 infection as a basis, Watanabe et al performed an exciting study focusing on the inhibition of activation of NF-κβ by dehydroxymethylepoxyquinomicin (DHMEQ), a derivative of epoxyquinomicin C.

Working with ATL-derived cell lines, the authors found that DHMEQ inhibits constitutive NF-κβ activity in ATLL-derived cell lines. The drug induces apoptosis of these cell lines by downregulating expression of antiapoptosis genes (Bcl-xL, Bcl-2, c-myc, cyclin D1, etc) and upregulating proapoptotic ones (caspase-3, -8 and –9). Moreover, the compound has antiproliferative effects, since an increase in cells in the G0/G1 phase after therapy with DHMEQ is observed in ATL cell lines. Fortunately, the incubation of control cell lines with DHMEQ showed no significant changes in the parameters studied. In a second step, working with fresh primary ATL cells from three patients, they also showed that DHMEQ significantly blocked NF-κβ DNA binding activities, reducing the viability of primary ATL cells. As in the control cell line experiments, peripheral blood mononuclear cells from control individuals were not affected by DHMEQ. In a model of severe combined immunodeficiency mice inoculated with an HTLV-1 transformed cell line, DHMEQ prevented the growth of tumoral cells and increased survival significantly compared with control animals not receiving DHMEQ. Finally, the authors explored the possible usefulness of DHMEQ in the purging of HTLV-1 cells as a preventive measure, since an increased proviral load is one of the known risk factors for the development of ATL. As HTLV-1 genes are not expressed in vivo, Watanabe et al searched for expression of the p65 NF-κβ component (typically observed in HTLV-1-infected cells) in lymphocytes that express IL-2Rα (regularly expressed on the surface of infected cells). This co-expression was observed in samples from HTLV-1 carriers, but not in controls. They then incubated PBMCs from HTLV-1 carriers with DHMEQ. As anticipated, they observed a reduction in the number of proviral copies, suggesting that DHMEQ can purge the peripheral blood mononuclear cells of HTLV-1 infected individuals.

In summary, the results presented in this paper suggest that constitutive NF-κβ activation plays a very important role in supporting survival of ATLL cells, making the use of DHMEQ a reasonable approach in controlling the disease. DHMEQ produce apoptosis in ATLL cells and derived cell lines, reduces the number of HLTV-1-infected cells (proviral load) in peripheral blood mononuclear cells, and prolongs the survival of immunodeficiency mice inoculated with an HTLV-1 transformed cell line, without apparent toxicity for normal uninfected-cells. Bortezomib, another NF-κβ inhibitor, now widely used against hematologic tumors, inconsistently inhibits ATLL proliferation. Other compounds seem less specific or produce an irreversible inhibition of NF-κβ (which may increase their toxicity in vivo). Therefore, DHMEQ seems the most promising compound of this group of drugs for ATLL therapy. The use of the drug to decrease proviral load also seems interesting and promising, although toxicity and pharmacokinetic studies are necessary before its use in clinical trials in ATL patients/HTLV-1 carriers.

Adoptive specific cellular immunotherapy

Viral and fungal infections, particularly CMV and *Aspergillus* spp, are still important challenges in the allogeneic-HSCT setting. Although an improvement in early diagnosis and therapy has been achieved in recent years, they remain an important cause of morbidity and mortality. Long-term prophylactic approaches, such as extended ganciclovir or intraconazole prophylaxis, are not exempt from complications. A specific T-cell response is basic for the control of CMV infections and prevention of CMV disease. It also seems to be an important defensive line against *Aspergillus*, although it has been less studied[46,47]. The use of adoptive specific cellular immunotherapy is an interesting alternative approach for the prevention and treatment of viral infections. In HLA identical HSCT, successful results have been published for CMV[24] and EBV[25]. GVHD, the most feared complication of cellular immunotherapy, does not seem to increase if pathogen-specific cells are given. HLA barriers have been considered a major obstacle for the spread of these therapies to the mismatch HSCT. The recent publication by the Perugia group[9] challenges this view.

Perruccio K, Tosti A, Burchielli E, Topini F, Ruggeri L, Carotti A et al. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. Blood. 2005;106: 4397-406.

The experience reported by the Perugia group is a masterpiece in the clinical development of immunotherapy. They performed their study in probably the worst scenario, the haploidentical HSCT patient, where an extraordinary susceptibility to infections is combined with a very high risk of GVHD. They described the first use of, and clinical response to, adoptive immunotherapy for CMV in haploidentical stem-cell transplants, and the first use of adoptive therapy for *Aspergillus* infection in any form of human hematopoietic cell transplantation. Specific T-cells were used without any post-transplant immunosuppression. In this scenario, it is known that the infusion of grafts containing only a few more T-cells than the threshold dose of 10^4/kg may cause lethal GVHD.

The aim of this study was to evaluate the efficacy and safety of specific nonrecipient-reactive T-cell clones in the prevention of CMV infection and in the treatment of invasive aspergillosis in adult haploidentical HSCT. Peruccio et al generated ex vivo specific CD4+ T-cell clones against CMV and *Aspergillus*, selecting those that were nonreactive against recipient cells. The specific nonrecipient-reactive T-cell clones were obtained after 30 days of complicated laboratory procedures from 200 ml of donor blood collected before G-CSF mobilization. Consecutive patients were randomly assigned to the immunotherapy or control groups. Only transplant recipients with evidence of invasive aspergillosis were recruited to the *Aspergillus* adoptive therapy trial. Regardless of assignment to the immunotherapy (n = 10) or control group (n = 13), all patients with evidence of invasive aspergillosis received liposomal amphotericin (6 mg/kg per day). In the CMV adoptive therapy trial, 27 received immunotherapy and 41 formed the control group. Between days 13 and 37 after transplantation (median 21 days), one infusion of clones was given to at least 3 recipients per cell dose. Doses started at 10^5 cells/kg and were escalated to 3×10^6 cells/kg.

The experience of the perugia group group shows that the infusion of ex vivo generated pathogen-specific CD4+ T-cell clones against CMV and Aspergillus that were nonreactive against recipient cells in haploidentical HSCT recipients who did not receive immunosuppression after SCT had the following results:

– High frequency T-cell responses were induced against these pathogens within 3 weeks of the infusion and they remained high for as long as they were monitored (36 weeks after transplantation). Despite the fact that the infusions were CD4+ T-cell clones, high frequencies of specific CD8+ T-cells were also detected.

– CMV reactivated less frequently in the adoptive immunotherapy group than in the control group (28% vs 90%) ($p < 0.004$). Furthermore, the total number of weeks of ganciclovir for positive CMV antigenemia was lower in the immunotherapy group than in the control group (2 vs 6.9) ($p < 0.005$).

– *Aspergillus* pneumonia tended to resolve more often among patients who received adoptive immunotherapy than in controls (90% vs 54%) (p 0.062). In all patients in the immunotherapy group, the galactomannan test became less than 1 ng/ml within 6 weeks of infusion compared with zero in the controls (p 0.002).

– CMV immunity after transplantation from naive donors into CMV-positive recipients can be achieved with the generation ex vivo of CMV-specific CD4+ recipient clones that are non-reactive with donor cells.

– Doses of specific T-cell clones $\leq 1 \times 10^6$/kg are safe. There was only one case of grade II acute GVHD in a patient who received 3×10^6 (anti-CMV) cells/kg.

What are the drawbacks of this approach? If the results are so impressive, why is this approach not extensively used, at least for standard therapy-resistant infections? First, the method is too complex to be used in clinical practice in most hospitals. Second, the ex vivo generation of pathogen-specific T-cells with this method takes several weeks, which complicates their use in patients in whom standard therapy is not successful. Many patients with severe infection might die while pathogen specific T-cells were being prepared in the laboratory. Third, it should not be forgotten that this experience, although randomized, is preliminary and the number of patients is relatively small. An increase in GVHD after T-cell infusion was not observed and a possible benefit in the management of these infections is suggested. Nevertheless, these results should be interpreted with caution. Finally, in Perruccio et al's series, due to other infection-related deaths among patients in the adoptive immunotherapy group, the advantage in cumulative incidence of overall infectious mortality was much less pronounced (0.53 vs 0.41 for the control and immunotherapy groups, respectively).

In summary, cloning of pathogen-specific T-cells seems a promising approach in the management of certain infections after HSCT. An increase in GVHD was not observed in this high-risk group of haploidentical HSCT recipients. Due to the delay in cloning T-cells and its methodological complexity, it is difficult to introduce this technique in the routine of most hospitals. Shortening the time of in vitro culture with more efficient culture techniques and collaboration with central laboratories are therefore essential for the introduction of this methodology in routine clinical practice.

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