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Review

Human antibody technology and the development of antibodies against cytomegalovirus

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ABSTRACT

Cytomegalovirus (CMV) is a virus that causes chronic infections in a large set of the population. It may cause severe disease in immunocompromised individuals, is linked to immunosenescence and implied to play an important role in the pathogenesis of cardiovascular diseases and cancer. Modulation of the immune system's abilities to manage the virus represent a highly viable therapeutic option and passive immunotherapy with polyclonal antibody preparations is already in clinical use. Defined monoclonal antibodies offer many advantages over polyclonal antibodies purified from serum. Human CMV-specific monoclonal antibodies have consequently been thoroughly investigated with respect to their potential in the treatment of diseases caused by CMV. Recent advances in human antibody technology have substantially expanded the breadth of antibodies for such applications. This review summarizes the fundamental basis for treating CMV disease by use of antibodies, the basic technologies to be used to develop such antibodies, and relevant human antibody specificities available to target this virus.

1. Cytomegalovirus – a clinical problem

Human cytomegalovirus (CMV) is a virus that commonly infects the human population. It belongs to the herpes family of viruses and it is estimated that 50–100% of the world's population have been infected with this DNA virus (Mocarski et al., 2007). Although CMV may affect healthy individuals and cause mononucleosis, fever, hepatitis and fatigue, this is rare, and mostly CMV causes a mild upper respiratory infection or infects its host without symptoms. After a primary infection CMV establishes latency and persistence; i.e. this virus is not eliminated by the host's immune system (Sinclair and Sissons, 2006). Like other members of the herpesvirus family, it remains dormant in cells in the body and asymptomatic reactivation occur throughout life (Sinclair and Sissons, 2006).

CMV is hence a common virus that has most likely evolved with humans since the early beginning of human life. By being an expert to survive in its host it has not created life-threatening conditions that would terminate the virus itself. Until the mid 1960s, CMV was not believed to cause any clinical pathologies, except for rare cases of severe congenital disease that was described for the first time in the 1890s (Weigert, 1898). In the 1960s, it was noted that CMV caused persistent fever episodes in patients receiving blood transfusions after open heart surgery and mononucleosis was also suspected to be caused by CMV in otherwise healthy individuals (Kaariainen et al., 1966; Klemola and Kääriäinen, 1965). Thereafter, large groups of immunosuppressed patients increased in our society with the development of organ and stem cell transplantation protocols in clinical medicine and the emerging epidemic of HIV. Among these patients who had a suppressed immune system, CMV was often reactivated and this virus rapidly became the most important cause of morbidity and mortality among transplant patients and AIDS patients in the mid 1980s (Britt and Alford, 1996).

At this time, CMV was diagnosed by tissue culture often taking 4–6 weeks, and limited treatment options were available for patients suffering from severe infections. Soon thereafter, PCR was developed and today most transplant centers monitor CMV reactivation and give prophylaxis against CMV, which have reduced life threatening CMV infections tremendously (Razonable and Paya, 2003). Ganciclovir, acyclovir and immunoglobulins containing CMV-specific antibodies were the first therapeutics to be used for this purpose and they reduced mortality of these infections, in particular those caused by CMV pneumonia. Despite these successes, the unmet medical needs in terms of prevention of CMV-induced diseases through vaccination and the diseases' cost to society (Stratton et al., 2001), also make a strong case for other, new disease-preventing options.

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Today the most important clinical diseases linked to CMV are congenital infections that represent the most prominent infectious cause of congenital malformations, hearing loss and learning disabilities, CMV retinitis in AIDS patients, and CMV syndrome, gastrointestinal disease and pneumonia in transplant patients. Among transplant recipients it was also noted that patients who have had CMV infections had higher incidence of other complications; deep bacterial and fungal infections, acute and chronic rejection in organ transplant patients, acute and chronic graft versus host disease in stem cell transplant patients, cardiovascular diseases and post transplant diabetes mellitus (Helantera et al., 2005; Hjelmesaeth et al., 2004; Kalil et al., 2003; Reinke et al., 1994; Rubin, 1989; van den Berg et al., 1996). These observations were made in epidemiological studies, and as the virus often was difficult to detect in the affected organ, these CMV associated pathologies were considered to be caused by indirect effects of this virus, e.g. by immune mediated mechanisms (Grattan et al., 1989; Lonnqvist et al., 1984). However, with more sensitive techniques, later studies have confirmed that CMV is indeed often present in kidney and heart grafts and associated with impaired graft function (Dzabic et al., 2011; Helantera et al., 2006). Thus, CMV likely causes harm also by direct effects in the affected graft with risk of later development of organ dysfunction and graft loss. Prophylaxis against CMV in organ transplant patients reduces the patients' risk of developing acute and chronic rejection, prolongs graft survival, and reduces other infectious complications and some malignancies (Kalil et al., 2003; Potena and Valantine, 2007; Valantine et al., 1999). Increased immunological control of CMV is also associated with reduced risk of rejection in heart transplant patients (Tu et al., 2006). Furthermore, although CMV is believed to be harmless in the healthy population, increasing evidence imply that it is associated with long-term consequences of immunosenescence (an inflation of the immune system) (Aiello and Simanek, 2012) and increased overall mortality. CMV is also implied in the pathogenesis of cardiovascular diseases and cancer (Aiello and Simanek, 2012; Gkrania-Klotsas et al., 2012; Simanek et al., 2011; Solana et al., 2012; Soroceanu and Cobbs, 2011).

1.1. Reactivation of latent CMV

The above findings led to the assumption that latent CMV was reactivated by immunosuppression as CMV was a clinical problem mainly in immunosuppressed individuals. However, it turned out to be the contrary. CMV establishes latency in myeloid lineage cells and it was not immunosuppression that led to reactivation of this virus; instead inflammatory activation of monocytes that differentiate into macrophages and dendritic cells allow for reactivation of latent CMV (Reeves et al., 2005; Soderberg-Naucler et al., 1997, 2001). If this happens in a patient with a suppressed immune system, clinical CMV disease may develop. As CMV infections often are associated with acute rejection or acute graft versus host disease, organ and stem cell transplant patients most likely trigger reactivation of latent virus by the inflammation caused by the activated immune system in these patients. In AIDS patients, CMV infections frequently developed in late stages of the disease, often after many other infectious problems that would also create a microenvironment conducive for reactivation of latent virus.

1.2. CMV infection in cancer

Already in the mid 1970s it was observed that patients with several cancer forms had a higher prevalence of CMV-specific antibodies and also higher antibody titers. At the same time Fred Rapp's group isolated a CMV strain that appeared to transform cells *in vitro* and when these cells were transferred to immunodeficient mice, tumors developed (Geder et al., 1976). This was against the dogma. CMV causes a lytic infection in most cell types and hence it should not be an oncogenic virus. Infection of cells arrests them in G1 phase that should also prevent oncogenic transformation. However, CMV encodes for many viral proteins that can promote tumor biology relevant mechanisms and under certain circumstances some viral proteins can transform cells (Soroceanu and Cobbs, 2011). Thus, CMV has developed sophisticated mechanisms that affect many different cellular and immunological functions, of which several are highly relevant for tumor biology (Soderberg-Naucler, 2006).

During the last decade, several groups have identified CMV DNA as well as CMV proteins in several human cancer forms (>90% of glioblastoma, medulloblastoma, neuroblastoma, sarcoma, mucoepidermoid cancer, breast, colon and prostate cancer) (Baryawno et al., 2011; Cobbs et al., 2002; Harkins et al., 2002, 2010; Rahbar et al., 2013; Samanta et al., 2003; Wolmer-Solberg et al., 2013); an infection that is absent in healthy surrounding tissues, indicating a tumor-relevance for this virus. CMV proteins are also found in 94–98% of sentinel lymph nodes (Taher et al., 2013) and in brain metastases of colon and breast cancer (Taher et al., 2015).

Although some researchers have failed to detect CMV in tumors, stimulation of dendritic cells with glioblastoma tumor lysates leads to expansion of CMV-specific T cells in glioblastoma patients, which provides indisputable immunological evidence that CMV peptide epitopes are indeed present in glioblastoma tumors (Prins et al., 2008). Furthermore, CMV-specific T cells directed against the pp65 protein can recognize and kill autologous glioblastoma cells (Nair et al., 2014a). Due to the fine-tuning of immune specificity, these observations suggest that CMV is clearly residing in glioblastoma cells. The high association with CMV and cancer therefore raises concerns that this virus may be an important player or even a cause of several cancer forms. This assumption is further supported by pre-clinical and clinical data demonstrating strongly suppressed tumor growth and indications of highly improved patient survival using anti-CMV targeted therapy. In animal models, anti-CMV treatment reduced medulloblastoma growth by 72% (Baryawno et al., 2011), neuroblastoma growth by 40% (Wolmer-Solberg et al., 2013) and reduced glioblastoma growth significantly (Hadaczek et al., 2013). Treatment of 50 glioblastoma patients who received anti-CMV treatment as an add-on to standard therapy at the Karolinska University Hospital as adjuvant treatment demonstrate a remarkably high survival: the 2 year survival was 70% among 40 patients receiving 6 months of anti-viral therapy and as high as 90% among patients with continuous treatment ($n = 25$) compared with 18% in contemporary controls ($n = 137$); median OS was 56.4 months compared with 13.5 months in the latter control group ($p < 0.0001$ (Soderberg-Naucler et al., 2013)). These observations call for a deeper understanding of CMV's role in cancer and of whether this virus is a novel target in anti-cancer therapy. The presence of CMV in glioblastoma would also imply that immunotherapy protocols that target CMV epitopes expressed in the tumor should be exploited as cancer therapy (Nair et al., 2014b; Schuessler et al., 2014). Several immunotherapy trials are currently under way to evaluate different CMV based protocols for glioblastoma patients, none however presently involving monoclonal antibody therapies targeting CMV.

1.3. Summary

There are multiple important clinical conditions in which CMV may play a significant role. Anti-viral therapy, including that based on immunological principles, may thus be a substantial clinical value in multiple settings. Efforts to develop such therapies, including those based on passive administration of highly effective monoclonal antibodies, the focus of this review, are thus worth while.

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