REVIEW

REACTIVATION OF LATENT DNA VIRUSES DURING IMMUNOSUPPRESSION: BASIC CONTEMPLATION

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Summary. – Immunosuppression (ISP) affects the outcome of acute virus primoinfection as well as the course of virus latency. ISP may result from viral infection of the immune cells. This infection can be either transient, as in measles virus infection, or progressive as in Human immunodeficiency virus (HIV-1, 2) infections, which proceed to acquired immunodeficiency syndrome (AIDS). Innate ISP occurs due to various congenital defects of cells, participating within immune response. In the majority of cases, ISP may be initiated by physical causes (e.g. irradiation) or by chemical substances including drugs used for post-transplantation therapeutic regimens. The hallmark of ISP is the impairment of B or T lymphocyte functions. Helper T lymphocytes produce a great variety of cytokines providing B lymphocyte proliferation and cytotoxic T cell maturation. The mature and/or activated memory cytotoxic T lymphocytes destroy the target cells, which synthesize virus-specific proteins. Since many ISP drugs hamper or downregulate the proliferation of B and T lymphocytes, their administration creates favorable conditions for the replication of reactivated DNA viruses otherwise produced in low amounts. Thus, ISP causes an extensive increase in the low-grade production of persisting virus, which might regularly occur during latency. In addition to reactivation of virus latency, ISP potentiates the replication and spread of any intercurrent infectious agent, whether bacterial, parasitic, yeast or fungal origin.

Key words: immunosuppression; organ transplantation; DNA viruses; reactivation; immune response

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Abbreviations: AIDS = acquired immunodeficiency syndrome; AP1 = activation protein 1; APC(s) = antigen-presenting cell(s); BKV = BK virus; CD = cluster of differentiation; CMV = cytomegalovirus; CPA = cyclophosphamide; CsA = cyclosporin A; DC = dendritic cells; ssDNA, dsDNA = single, double stranded DNA; EBV = Epstein-Barr virus; EBNA = EBV nuclear antigen; ER = endoplasmic reticulum; HBV = Hepatitis B virus; HBcAg = hepatitis B core antigen; HBeAg = hepatitis B "e"antigen; HBsAg = hepatitis B surface antigen; HCV = Hepatitis C virus; HHV-6,7 = Human herpesvirus 6,7; HIV-1, 2 = Human immunodeficiency virus 1, 2; HSV-1, 2 = Herpes simplex virus 1, 2; ICAM = intercellular adhesion molecule; IE = immediate early; IF = immunofluorescence; IFN = interferon; IL = interleukin; IM = infectious mononucleosis; IRF = interferon regulating factor; iRNA = intermediate RNA; ISP = immunosuppression; Jak = janus kinase; JCV = JC virus; KS = Kaposi's sarcoma; KSHV = Kaposi's sarcoma-associated herpes virus; LANA = latent nuclear antigen; LAT = latency associated transcript; LMP = latent membrane protein; MHC = major histocompatibility complex; MIP = macrophage inflammatory protein; MMF = mycophenolate mofetil; mRNA = messenger RNA; NFκB = nuclear factor *kappa* B; NK = natural killer (cells); PEL = primary effusion lymphoma; PKR = phosphokinase R; PML = progressive multifocal encephalopathy; PTLD = post-transplantation lymphoproliferative disease; STAT = signal transduction and activation of transcription; TAP = transporter antigen processing; Tc = cytotoxic T lymphocyte; Th = helper T lymphocyte; TCR(s) = T cell receptor(s); TIR = Toll interleukin receptor; TLR = Toll-like recptor; TNF = tumor necrosis factor; vDNA, vRNA = viral DNA, RNA; VZV = Varicella-zoster virus

Contents:

1. Viruses and the immune system

1.1. Introduction

DNA viruses comprise a large group of infectious agents from 20 to 300 nm in size (Fauquet *et al.,* 2005). Similar to any virion, the DNA viruses pass 0.45 nm bacterial filters and are not visible in the light microscope (Hsiung *et al.,* 1982). With the exception of families *Parvoviridae* and *Circoviridae*, which contain single stranded DNA (ssDNA), all other DNA viruses contain double stranded DNA (dsDNA). Their genome is located within the capsid, which protects the genome from environmental damage. The capsids of naked (unenveloped) viruses also provide other functions, such as attachment to the cell surface, penetration and transfer of the capsid content across cell membrane. The enveloped viruses are wrapped up in a lipid membrane containing viral glycosylated proteins. HSV virions possess a structureless proteinacious tegument, located in between the inner capsid and outer envelope.

The virions attached to the surface receptors of susceptible cells are engulfed into endocytic vesicles. Later on, the DNA virus genome released from the virion – as result of uncoating – penetrates into cytoplasm and then (with exception of poxviruses) enters the nucleus. Transcription of viral DNA starts within the nucleus of infected cells. The viral mRNAs are transported to cytoplasmic ribosomes. The translation products that include capsid proteins, are transported back into the nucleus where they get selfassembled. Both dsDNA strands become templates for copying the new complementary viral DNA (vDNA) strands. Cellular as well as virus coded non-structural proteins may participate in vDNA replication by providing the components for the vDNA polymerase complex. The new vDNA is incorporated into nascent capsid within the nucleus. In the case of herpesviruses, the cellular as well as nuclear membranes are loaded with virus-coded glycoproteins. First, the capsid is enveloped at the nuclear membrane and then is transported to cell surface through a system of cytoplasmic vacuoles.

In the infected host, virions frequently attach to the epithelium cells of nasopharyngeal mucosa, to epidermal cells, or to cylindric epithelium cells of the respiratory and gastrointestinal tracts. The number of virions, released from the cells, which had been first infected usually exceeds many thousand (even million) times the number of virions present in the inoculated material. Newly produced virions are spread to the neighboring cells or transported to the regional lymph nodes. In addition, the virions are disseminated via blood (viremia) and/or they spread along nerves. Viremia and neural spread are essential for transmission of virions into target organs such as lungs, skin, liver, heart muscle, neural ganglia and brain. The involvement of target organs is the basis of clinical disease. A great proportion of new virions can be released from the body to ensure spread of the virus within human and to animal populations. Several diseases caused by DNA viruses occur at the time of reactivation of the latent virus harbored in the host from past acute infection. It was confirmed that the viral DNA of herpesviruses may survive during latency in a covalently circularized form mainly outside the chromosomes. Non-productive latency resides in neurons, glial cells, white blood cells and/or in certain epithelium cells (kidney, salivary glands, prostata). The molecular basis of latency relays of various mechanisms. In the case of HSV-l/2, latency associated RNA transcripts (LAT) are synthesized from a limited area of the genome, just close to the immediate early (IE) transcription region. Their function is still unclear, but they may represent silencer RNA molecules. A quite different model of latency in Epstein-Barr virus (EBV) infection means that IE mRNAs are made from a limited number of latency-associated genes, which become expressed as latency-associated proteins. There are several possibilities how reactivation occurs ranging from low level expression of at least one IE protein, through the expression

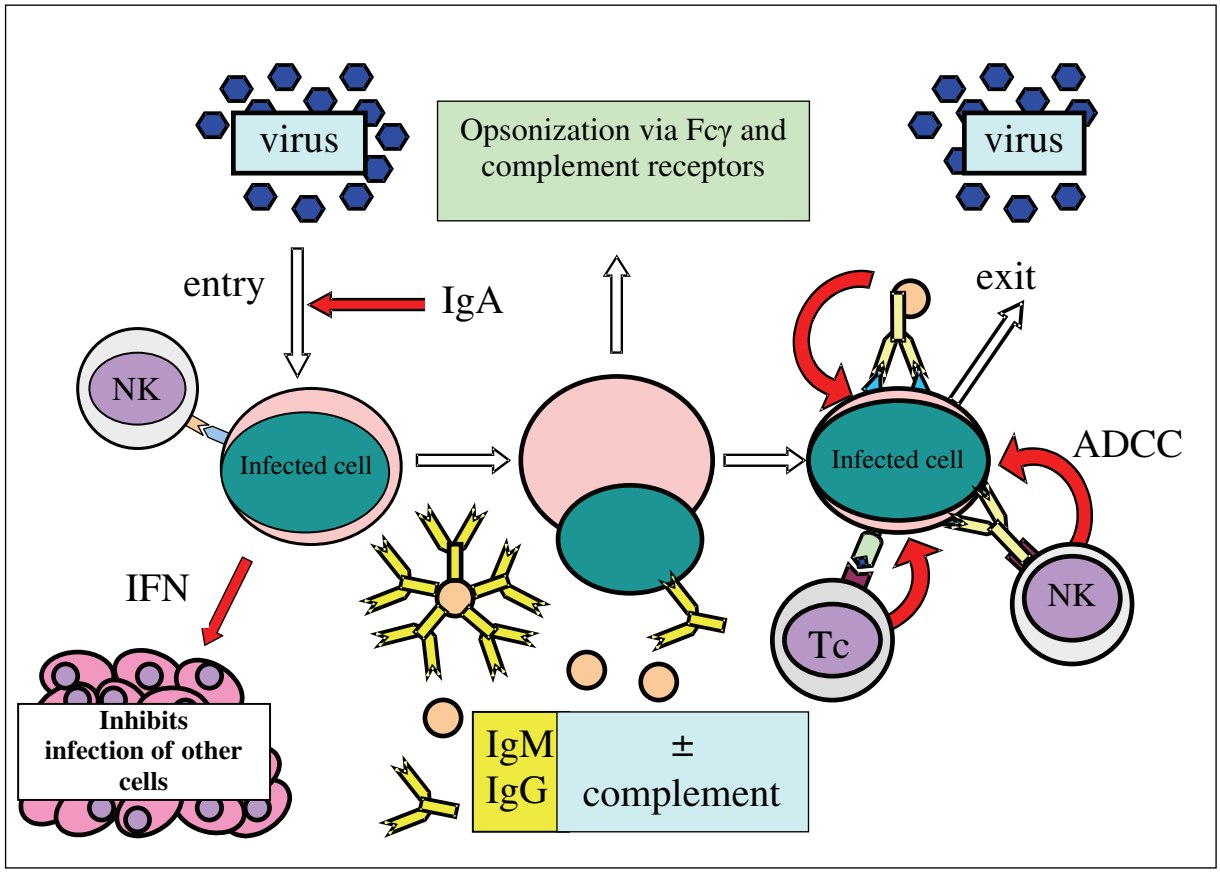

Fig. 1

Basic mechanism of the immune response in virus infections

Infected cells release IFN-I to protect other (non-infected) cells. Destruction of infected cells is mediated by NK cells, cytotoxic T lymphocytes, and by antibody and complement mediated cytolysis. The virus entry into cells may be prevented by IgA, IgG, and IgM antibodies that neutralize infectious virus. ISP acts mainly on Th and B cells, which indirectly affects the maturation of Tc lymhpocytes and the antibody level. ADCC = antibody dependent cell mediated cytotoxicity

of several transactivator and/or cell transformation proteins, to the production of minimal amounts of complete infectious virions. Under conditions of ISP, the latter situation favors the production of larger amounts of virions associated with inapparent virus shedding. If the reactivated virus replicates more extensively, the apparent clinical disease occurs.

Viruses are good immunogens inducing non-specific as well as specific humoral and cell-mediated immune responses closely associated with inflammation and cytokine formation. The systemic immune response is characterized by the presence of T lymphocytes equipped with virus-specific T cell receptors (TCRs), which recognize viral antigenic peptides associated with major histocompatibility complex class I (MHC-I) molecules present on the surface of antigen-presenting cells (APCs) and/or virus-producing target cells. The local immune response is mediated by non-specific inflammation, interferon (IFN) formation, by production of a class IgA secretory antibodies (sIgA) and by the accumulation of specific helper T lymphocytes in regional lymph nodes. The immunogenic capsid proteins and envelope glycoproteins possess antigenic peptides, which interact with the antigen binding sites of antibodies either soluble or B lymphocyte bound. The antigenic domains recognized by antibodies are different from the antigenic peptides recognized by the peptide-binding clefts of TCRs. In addition, certain non-structural virus proteins may induce a specific immune response. This is of great practical importance for signaling the onset of virus replication during reactivation of latency, preceding the production of complete infectious virions. The basic mechanisms how the immune response overcomes virus production are depicted in Fig. 1.

1.2. Innate immunity at early stage of virus infection

The lipid membranes wrapping the enveloped viruses and the double stranded viral intermediate RNA (iRNA) formed in the course of the RNA replication, interact with the Toll-like receptors (TLRs) present on macrophages and on epithelium cells. The double stranded iRNA is known as the most potent IFN type I (IFN-I) inducer. In association with HSV-1, 2 infections, the natural IFN-I producer cells are activated by TLR-9 (Lund *et al.,* 2003; Hochrein e*t al.,* 2004; Krug *et al.,* 2004). IFNs-I produced in infected cells represent a mixture of polypeptides encoded by different genes (leukocyte IFN-α and/or fibroblast IFN-β). The transcription of IFN genes is regulated by the family of interferon regulating factors (IRF), from which IRF3 is the most frequent (Nguyen *et al.,* 1977). IFN-I interacts with the receptors on the surface of neighbor cells, which become resistant to virus replication. Following this interaction, phosphorylation of signal transducer proteins (Janus kinase, Jak) activates the transcription of IFN-responsive genes by means of transcription activators such as STATl and/or STAT2 (signal transduction and activation of transcription) translocated into nucleus (Darnell *et al.,* 1994; Stark *et al.,* 1998). The STAT transcription factors recognize the IFN signaling responsive element sequence within the promoters of protein kinase R (PKR) and/or the 2-5A synthase (Biron and Sen, 2001). PKR phosphorylates the translation initiation factor eIF-2 to interfere with the formation of the translation initiation complex; this affects cellular metabolic events and results in the inhibition of virus replication (Williams, 1999). The 2, 5´-oligonucleotides are poly-A oligomers (pppA2-A5), which inactivate the mRNA leader sequence by an antisense acting mechanism. They also induce various RNase activities cleaving the vRNA (Rebouillat and Hovanessian, 1999).

In addition, IFN-I activates NK cells but also increases the expression of MHC-I molecules promoting the virus-specific cytotoxic T lymphocyte (Tc lymphocyte) mediated destruction of infected target cells. NK cells comprise a population of CD16/CD56 positive but CD3 and TCR negative lymphoid cells, which cytoplasm is rich of granules containing perforines and granzymes. NK cells do not undergo thymus independent selection. On the other hand, they express several non-specific receptors such as Fc-receptors, the C-lectin receptors (CD94/NKG2) and killer cell Ig-like receptors described in humans (Lanier, 1988). The latter interact with the MHC-I molecules type A, B, C and G (Rajagopalan and Long, 1999), while the former recognize the MHC-I molecules E (Braud et al*.,* 1998). The killer cell ig-like receptors recognizing the MHC-I molecules suppress the activity of NK cells by a feedback mechanism. If the number of MHC-I molecules is low or target cells are infected by viruses interfering with MHC-I expression, at that time NK cells preferentially cause target cell destruction. This "missing self" hypothesis offers a very attractive explanation for MHC-I-independent cytolysis (Ljunggren and Karre, 1990). As mentioned, the activity of NK cells predominates before the development of virus-specific CD8 Tc cells. In the presence of specific antibodies interacting with virus-specific glycoproteins transported to the surface of infected cells by means of their signal sequence, NK cells help immune cytolysis. Summing up, the activity of NK cells peaks at early stages of immune response and/or under conditions of impaired expression of MHC-I molecules due to virus-mediated interference.

Recent evidence indicates that certain TLRs, such TLR2 and TLR4 are essential for the initiation and production of earliest cytokines, which modulate the early (innate and non-specific) immune response. Several enveloped viruses, for example the respiratory syncytial virus (Kurt-Jones *et al.,* 2000), Coxsackie B4 virus (Triantafilou and Triantafilou, 2004), retroviruses (Rassa *et al.,* 2002), Human cytomegalo-virus (HCMV, Compton *et al.,* 2003) and HSV (Kurt-Jones *et al.,* 2004) induce production of cytokines such as interleukin (IL)-12, IL-1, IL-4, TNF and IFN-γ due to TLR stimulation. The cytoplasmic portion of TRL shares a common domain with the IL-1 receptor (IL-1R) labeled as Toll/IL-1R (TIR) motif. The TIR domain mediates the activation of nuclear factor *kappa* B (NFκB) and/or AP1 transcription factors resulting in their translocation to nucleus (Sandor and Buc, 2005). IL-12, IL-1 and IL-4 are produced at early intervals p.i. and may be associated with the rapid onset of clinical signs as fever, headache, muscle pain and exhaustion (Vasalli, 1992). The activation of TLR and NK cells by IFN-γ aims to mount a potent non-specific response until the specific immune T cells occurs. Non-vaccinated and/or non-immune individuals are relatively slow at mounting specific responses; satisfactory levels of virus-specific IgM antibodies appear within 3–4 days, while the highly specific IgG antibodies and Tc lymphocytes are present on day 7–10 p.i. (Butz and Bevan, 1998; Murah-Krishna, 1998). It is well-known that the early stage of illness ends by "a crisis" followed by the relief of acute clinical signs usually on day 5 from the onset of clinical symptoms. When the levels of specific antibodies rise and the specific Tc lymphocytes become abundant, the probability of infectious virus isolation decreases. Nevertheless, there is still feasible to attempt the detection of viral genome by PCR and/or to detect viral antigens in the body fluids by ELISA.

1.3. Virus-specific immune response

The specific immune response is closely associated with proliferation of helper T lymphocytes (Th lymphocytes), maturation of Tc lymphocytes and the formation of specific

antibodies. The early processing of viral antigens is mediated by mononuclear phagocytes (macrophages) and dendritic cells (DC) commonly called antigen-presenting cells (APCs). The APCs cleave the engulfed viral proteins into antigenic peptides that are presented at their surfaces by MHC-II molecules. The MHC-II-bound antigenic peptide is recognized by the TCR of Th lymphocyte. Following this interaction, the Th 2 cells start producing cytokines such as IL-4, IL-5, IL-6, IL-13 and IL-10 promoting antibody production, and Th 1 cells start to synthesize IL-2, IL-12, TNF and IFN-γ promoting Tc lymphocyte differentiation (Mossman and Cofman, 1993; Kelso, 1995).

The virus-coded proteins synthesized *de novo* on the ribosomes of virus-infected cells may be either cleaved into antigenic peptides or serve for the virus replication and assembly. One should realize the dual fate of virus-coded proteins, which either participate in viral replication cycle or are processed into antigenic peptides. Enveloped viruses encode polypeptides, which possess signal peptides of their own; such polypeptides are transported into endoplasmic reticulum (ER) and glycosylated within the Golgi zone. In contrast, the virus-coded proteins cleaved into antigenic peptides interact with transporter proteins associated with transporter antigen processing (TAP) and then are transferred to ER. Here they interact with the MHC-I molecules and are transported to the surface of infected cell. The MHC-I-bound viral peptides present on the infected cell surface are recognized by TCRs of Tc lymphocytes provided that both are syngenic in structure, what is controlled by the CD8 protein of Tc lymphocytes. The selection and maturation of specific Tc lymphocytes from their naive precursors starts during interaction with DC, which represent a special subpopulation of APCs expressing not only MHC-II, but also MHC-I molecules. The "cross presentation" of exogenous antigenic peptides to Tc lymphocytes of MHC-I molecules on the surface of DC is usually supported by interaction of co-receptors such as the B7 ligand (on DC) and the co-receptor CD28 (on Tc lymphocytes). The activation and proliferation of mature Tc lymphocytes goes together with excessive IL-2 production (Foy *et al*., 1994; Hollenbaug *et al*., 1994).

The Th cells that are equipped with a virus-specific TCR, have been selected by recognizing the MHC-II-bound antigenic peptides processed within APCs. The CD4 molecule of Th lymphocyte interacts with the invariable region of the MHC-II molecule. Additional signal for Th lymphocyte maturation and proliferation is coming from the interaction of its CD28 receptor with the B7 ligand of APC (Robey *et al.,* 1995; Whitmire and Ahmed, 2000). Activation of Th lymphocytes needs a tight interaction of the leukocyte function antigens (LFA) on Th cell surface (i.e. LFA-1 or LFA-2/CD2) with intercellular adhesion molecules (ICAM-1) and/or LFA-3 on the APC surface. The Th cells use the ligand CD40L to bind to the CD40 receptor of APC. The activated Th lymphocytes start to express the receptor for IL-2 (IL-2R) and to secrete soluble IL-2. This assures proliferation of the specifically selected Th lymphocytes (with TCRs recognizing the "correct" antigenic peptide). In addition, B cells interact with Th lymphocytes using similar ligands and receptors (see below).

Neither the matured antigen-specific Tc lymphocytes nor the memory Tc lymphocytes require co-stimulation via CD28 receptors. These Tc lymphocytes are ready to proliferate and to destroy the target cells equipped with MHC-I-bound antigenic peptides, as their TCR recognizes the specific antigenic peptide. Before exerting cytotoxic activity, the Tc lymphocyte must be tightly associated with the virus-infected target cell by means of adhesion ligands, such as LFA-1, LFA-2/CD2 interacting with ICAM of target cells. The cytoplasm of mature Tc cells contains a pore forming protein (perforin) and granule associated enzymes (granzymes). Perforin has a structure and function similar to the C9 component of complement. In the presence of Ca^{2+} its monomeric subunits are incorporated into the lipid membrane getting assembled into ring shaped pores. Thus, perforin forms openings within the target cell membrane allowing diffusion of granzymes from into its cytoplasm (Yewdell and Bennink, 2002). Granzymes are mainly serine proteases, which activate the intracellular cascade of apoptotic enzymes. The Tc cells also produce TNF and express a cytokine-like ligand, called *Fas* ligand (FasL). These chemokines act on corresponding receptors on target cells (TNFR and/or Fas/APO-1, respectively). The latter signals finally induce apoptosis leading to destruction of the target cell (Suda and Nagata, 1994). TNFR-1 is expressed on the surface of many cells with the exception of erythrocytes. Both, TNFR and *Fas*-receptor possess a cytoplasmic domain, which associate with the death domains of intracellular adaptor proteins, which activate the effector caspases (Shresta *et al.,* 1998; Zychlinski *et al.,* 1991).

The Tc lymphocytes recognizing the MHC-I-bound antigens in a specific manner play a pivotal role in recovery from virus infections. They appear at the site of virus replication within 3–5 days, i.e. before the onset of efficient specific IgG response. The virions are released from infected cells either by means of cellular mechanisms exploited by the infecting virus, or due to CPE induced by the virus, following cell-mediated cytotoxicity and/or due to antibody and complement mediated cytolysis. Since antibodies cannot act on the intracellular virus, cell-mediated cytolysis (either specific or non-specific) is of great importance for antiviral defence. Virus-coded nonstructural proteins, which interfere with the immune defence, act at various stages of the MHC-I-restricted cytotoxic response.

Antibodies comprise an extracellular (humoral) barrier against the spread of the free virus. To promote the

maturation and selection of B cells secreting specific antibodies, Th cells present the TCR-bound antigenic peptide to MHC-II molecules on the surface of B cells. In the next step, Th2 lymphocytes use the CD40 ligand (CD40L) to interact with the CD40 co-receptor of selected B cells (Kopf *et al.,* 1999; Murata *et al.,* 2000). Th 2 type cytokines IL-4, IL-13, IL-5 and IL-6 are produced that stimulate the proliferation of selected B cells to secrete specific antibodies. Stimulated B cells are converted into the plasmatic cells or become the specific memory B cells.

Virus-specific IgM and IgG antibodies secreted by activated B cells or plasmatic cells interact with the antigenic domains of virions. The coating of virions by antibodies is the basis for virus-neutralization, since virion-bound antibodies prevent the adsorption of virus particles to susceptible cells. The formation of virion-antibody complex is facilitated by the presence of complement. In addition to the neutralization of free virions, the presence of the complement causes virolysis, especially in the case of enveloped viruses. Furthermore, the neutralized virions are better targets for phagocytosis (opsonized virions), since macrophages are equipped with Fc receptors. Finally, antibodies interact with the surface of infected cells, which express virus-coded glycoproteins. The destruction of infected cells is accomplished by the virus-specific antibodies associated with complement or by non-specific killer cells (K cells). Cytolysis of infected cells by K cells in the presence of virus-specific antibodies is called antibody dependent cellular cytolysis.

1.4. Mucosal immunity

Mucosal immunity develops as a local imune response related to the mucosal surface – mucosa associated lymphoid tissue. Lymphatic tissue lines the respiratory airways, the gastrointestinal and urogenital tract, the middle ear, the conjunctival and nasopharyngeal mucosa and surrounds the salivary, lacrimal and mammary glands. The lymphatic cells and APCs are dispersed in the midst of epithelium cells and close to their basement membrane. Relatively large uncapsulated follicles form Payer plaques in ileum submucosa; their aggregates comprise the appendix and tonsils. The lymphatic tissue within intestinal wall is covered by the cylindric epithelium containing microfold cells. These cells transport large uncleaved proteins and their corpuscular aggregates not excluding viruses into *lamina propria.* Viruses easily reach macrophages and other APC elements as well as submucosal B cells to initiate various forms of immune response. The virions, which replicate within the epithelium cells of mucosal surface come into contact with DC located directly among the spinous epithelium cells and with intraepithelial Th cells. In response to antigen stimulation of B cells, antibodies of the IgA and IgM class are produced directly within mucosa and/or submucosa. The monomeric IgA molecules mainly diffuse into blood, while the dimeric IgA molecules are transported across the epithelium cells, where they become associated with the IgA glycopolypeptide called secretory piece. The final secretory s-IgA complex covers the respiratory and digestive tract mucosa and is secreted into saliva, tears, and maternal milk. Furthermore, the intestinal wall contains a subpopulation of T lymphocytes, termed γ/δ T cells, which are thymus independent. The γ/δ T cells recognize viruses via alternative receptors other than MHC-I molecules (Yewdell and Benninck, 2002). Similarly to NK cells, the γ/δ T lymphocytes mount a non-specific immune response and they downregulate the cytokine secretion in CD3 T lymphocytes by a feedback mechanism.

2. Immunosuppressive drugs and immunossuppression (ISP)

In general, ISP means impaired function of immune system caused by physical, chemical and/or biological means. X-rays, ultraviolet light A and γ-radiation are the best examples of physical agents exerting ISP effect. ISP is an undesired side effect elicited by antimetabolites such as cyclophosphamide or mytomycin C (alkylating substances), cytosine arabinoside and 5-fluorouracyl (pyrimidine analogues), metothrexate (folic acid analog), imuran (azathioprine) and the so-called cytotostatic antibiotics (actinomycin C, doxorubicin, bleomycin, vincristin etc). Typically, these substances are used in the cancer therapy. The classical ISP drug cyclophosphamide (CPA) is converted into an active metabolite within the liver. It inhibits proliferation of B as well as T lymphocytes not excluding other hemopoetic cells (Makinodan *et al.,* 1970). As a result, CPA causes decreased antibody formation. The effect on cell-mediated response depends of the timing of CPA administration as related to virus administration (Meta *et al.,* 1977). Due to its action, CPA is rarely used in the post-transplantation ISP regimens. Azathioprine (Aza) causes accumulation of 6-thioguanine in the patient's DNA, an effect analogous to that of ultraviolet radiation A. Therefore, Aza-treatment may be associated with selective photosensitivity and higher prevalence of skin cancer (O'Donovan *et al.,* 2005). To avoid non-selective side effects, monoclonal antibodies reacting with the CD20 marker of B cells were developed for the therapy of non-Hodgkin lymphoma (Rituximab, Ibritumomab, Tositumomab) and/or antibodies reacting with epidermal growth factor receptors are recommended in breast and colorectal carcinomas (Cetixumab, Erbitux, Trastuxumab).

Macrolide antibiotics (cyclosporin A (CsA), FK506, rapamycin), mycophenolate mofetil (MMF), morpholino-

ethyl ester of mycophenolic acid (Solinger, 1995) are specific ISP drugs used in post-transplantation ISP therapy. All the macrolides are cyclic oligopeptides inhibiting signal transduction pathways within T lymphocytes (Ho *et al.,* 1996). CsA, FK506 (tacrolimus) as well as rapamycin associate with cyclophilin. At a relatively high dose, CsA may impair several macrophage functions, such as macrophage disappearance reaction, IL-1 generation, prostaglandin E production and chemotaxis (Matsushima and Baba, 1990). The FK506/cyclophilin complex inhibits a Ca^{+2} regulated serine-threonine phosphatase, called calcineurin (Schreiber and Crabtree, 1992). Inhibition of calcineurin results in a complete inactivation of the nuclear factor of activated T cells. There is no expression of CD40L, IL-2 and IL-4 needed for proliferation of Th as well as B cells. In the presence of the macrolide calcineurin inhibitors, Th cells fail to fulfill their helper functions in direction of B lymphocyte expansion as well as in Tc lymphocyte proliferation. Rapamycin also interacts with cyclophilin, but later on targets a regulatory kinase, originally termed as molecular target of rapamicin (mTOR) (Abraham and Wiederrecht, 1996). Rapamycin inhibits the IL-2 induced proliferation of T cells causing their arrest in the G1 phase. The growth arrest is characterized by the presence of the catalytically inactive cyclinE/cdk2 complexes. Rapamycin can reduce the frequency of post-transplant lymphoproliferative disorders elicited by EBV, namely by inhibiting viral IL-10 secretion (Nepomuceno *et al.,* 2003). Decreased IL-10 production is accompanied by blocking of the transcription factors STAT1 and STAT3 (signal transcription and activator of transcription) promoting B cell growth. Thus, rapamycin is a promising drug for treatment of post-transplantation EBV-associated B cell lymphomas, while simultaneously providing the prevention of graft rejection. The non-nephrotoxic anti-proliferative agent MMF is a dehydrogenase inhibitor blocking the synthesis of guanosine monophosphate (Inouye *et al.,* 2002). It has been found to represent a potent ISP drug, when combined with CsA and steroids (MMF study group, 1995; 1996). MMF can be also combined with antilymphocyte globulin (Grinyo *et al.,* 1998) or cortico-steroids (Eckberg *et al.,* 2006). Since MMF enhances the effect of cytidine analogs (lamivudin), thymidine analogs (zidovudin), and purine analogs (didanosine), its pontential use for supportive treatment of HIV-infected patients is under investigation.

Biological agents having ISP effect are certain hormones (glucocorticoids, cortisol), and specific antisera reacting with CD3 of T cells [i.e. anti-thymocyte globulin, humanized monoclonal antibody acting against the IL-2R/CD25 of the matured T lymphocytes (Daclizumab) and humanized monoclonal antibody anti-CD52 (Alemtuzumab)]. Summing up, the common feature of ISP drugs is their ability to impair the functions of Th lymphocytes, such as activation, stimulation and/or expansion. An important effect of medical ISP is the decrease of specific antibody production and the blockade of Tc lymphocyte activation.

3. Virus interference with the immune system

HIV-1, 2 are the best-known example of the viruses causing ISP. Envelope glycoprotein gp120 of HIV-1 interacts with the CD4 molecule of Th lymphocytes (Dalgleish *et al.,* 1984). Alternative HIV receptors were identified too, e.g. the chemokine receptor CCR-5 (Wu *et al.,* 1996). During acute replication, HIV destroys the host Th cells while during latency the DNA copy of vRNA remains integrated in host cell chromosomes (Freed and Malcolm, 2001). The main criterion of progression from latent HIV-1 carrier state to AIDS is the decrease of the number of Th lymphocytes within blood (<200/μl), which is indirectly related to the level of vRNA (viremia) in blood plasma (Guatelli *et al.,* 2002). During AIDS several latent viruses reactivate such as HCMV, human herpesviruses 6 and 7 (HHV-6, HHV-7), human polyomaviruses (BK virus, JC virus). Probably the most dangerous virus that reactivates with association of the HIV-1 infection is the Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8). Under conditions of HIV-1 reactivation, the simultaneously reactivated KSHV causes the invasive form of Kaposi's sarcoma (KS). Originally, KS was described as a chronic slowly progressing skin disease with sarcomatous vascular proliferation (Craighead, 2000a). The invasive form of KS involves the lymph nodes and the wall of gastrointestinal and tract (Hirsch and Curran, 1990; Moore and Chang, 2002). In addition to generalization of the reactivated cytomegalovirus (CMV), the impaired function of Th cells causes dissemination of miliary tuberculosis, severe forms of parasitic infections (Cryptococcus meningitis, generalized toxoplamosis) and/or widespread fungal diseases (pneumocystic pneumonia, candidosis).

The AIDS syndrome is an example of infectious disease occurring in the individual failing to have the cell-mediated immune response due to elimination of CD4 T lymphocytes. The blockade and impairment of Th cells due to iatrogenic ISP (post-transplantation therapy) may cause similar problems, but usually with less dramatic course. The relative "advantage" of iatrogenic ISP is the possibility to interrupt the drug administration or to use a less drastic ISP regimen. The most frequent situations occurring in the course of ISP treatment were studied experimentally (Table 1). During acute primoinfection, ISP increases the replication rate as well as the duration of productive virus growth. The conditions favoring virus spread from the portal of entry may increase the lethality of acute infection, especially in

Table 1. Basic effects of ISP in virus infections

Timing	Result
ISP applied during the incubation period (following administration of sublethal virus dose or of a virus showing low-grade virulence).	Increased lethality resulted from acute illness.
Administration of a non-pathogenic and/or non-cytocidal virus during ISP regimen.	High amounts of virus produced in the absence of acute disease; immunological tolerance may develop. Establishment of persistent infection; late clinical signs occur due to antigen-antibody deposits.
ISP starts following the establishment of latency.	Reactivation of the virus occurs at higher frequency and has a more severe course manifested by clinical disease.

Table 2. DNA virus interference with the immune response (part 1)

Virus	Mechanism of action	Protein
HCMV	Degradation of MHC-I and II molecules	US2
	Interference with insertion of MHC-I into the ER membrane	US3
	Inhibition of TAP (interferes with the peptide transport to ER)	US6
	Interference with intracellular signaling (G-protein homolog)	US27
	Chemokine receptor MIPα homolog	US28
	Inhibition of NK cell-mediated cytolysis	UL18, UL40
	Interference with the production of Th1 type cytokines	UL111A
	TNFR (TNF receptor) homolog	UL144
	CXC chemokine antagonist	UL146
	Chemokine antagonist (precise effect unknown)	UL147
KSHV	Inhibition of IFN-α/β induction, viral IRF	K9
	CCR8 (chemokine receptor homolog)	vMIP-1
	CC, CRC and CXC chemokine antagonist	vMIP-2
	B cell and endothelium cell proliferation factors	vIL-6/K2
	Apoptosis inhibitor, caspase 1 blocking protein	vFLIP/K13*
Gammaherpesviruses	Apoptosis inhibitor	vBcl-2/ORF74

*vFLIP = *Fas*-associated death domain like interleukin 1β converting enzyme inhibitor protein

Table 3. DNA virus interference with the immune response (part 2)

Virus	Mechanism of action	Protein
Human papillomavirus	Inhibition of apoptosis	E6
	Inhibition of antigen presentation by MHC-II molecules	E5 and E6
Human adenovirus	Inhibition of apoptosis (*Bcl-2* analog)	E1B-19K
	Inhibition of apoptosis (p53 inactivator)	E1B-55K
	Destruction of *Fas*-receptor	E3-10.5/14.5K
	Inhibition of TNF	E3-10.5/14.5K
	Inhibition of IFN-α/β action	E1A
HSV-1, 2	Inhibition of TAP (interferes with the peptide transport to ER)	IE protein ICP47/US12
	Interferes with PKR, reversion of IFN-α/β action	ICPγ34.5
	Inhibition of TAP	US3
	PKR binding, reversion of IFN-α/β action	US11 (acts along with ICPγ34.5)
	Binding of complement C3	Envelope gC
	Interacts with IgG via the Fc-binding domain (FcR homolog)	Glycoprotein E and I complex
EBV	Inhibition of antigen presentation by MHC-II	BZLF2 gene product
	Binding of colony stimulation factor	BARF1 gene product
	Interference with the production of Th1 type cytokines	Viral IL-10 homolog, BCRF1 gene product
	PKR inhibition, reversion of IFN-α/β action (unclear mechanism)	EB early RNA (EBER)
	Inhibition of IFN-α/β responsive transcription	EBNA-2
	Inhibition of apoptosis (*Bcl-2* production)	LMP-1

the case of less virulent viruses. If applied prior to virus inoculation, ISP increases the susceptibility of the host to infection, which then shows a more aggressive course. Lastly, the virus that had become latent before ISP more frequently reactivates. If ISP continues, the reactivated virus would replicate in uncontrolled manner causing more severe signs of disease as compared to other reactivation stimuli.

Many DNA viruses encode non-structural proteins, which interfere with the virus-induced immune response (Table 2 and 3). Well-known examples are EBV infecting B lymphocytes, the KSHV infecting B lymphocytes and fibrocytes, and the HCMV, infecting T lymphocytes, macrophages and certain epithelium cells. When DNA viruses interact with the lymphocytes in a non-productive manner, the presence of virus-coded proteins may impair antigen processing by blocking MHC-I or TAP molecules. Among the RNA viruses other than HIV that affect the functions of immune system, a good example is the measles virus, infecting T lymphocytes and APC. In general, measles virus causes a transient ISP (Oxman, 2002). During its replication in macrophages, the expression of LFA1 increases due increased adhesive properties. The measles-infected macrophages adhere to capillary endothelium cells especially close to the basement membrane of skin epidermal cells. The virus spreads from the capillaries into the epidermis causing typical morbilliform exanthema. The glycoprotein H of measles virus interacts with the CD46 molecules on the surface of T lymphocytes increasing their susceptibility to complement mediated cytolysis (Schneider-Schaulis *et al.,* 1996). Even when no T cell lysis occurs, the intracellular signaling against any external antigen is impaired (Aota *et al.,* 2001). The transient paralysis of T cell response in measles infected host abolishes the existing cell-mediated responses for several months. Another kind of lymphocyte alteration in due to myxovirus infections is characterized as the defect of migration (Snodgrass *et al.,* 1972). Influenza virus affects the traffic CD3 T cells causing their depletion in the paracortical zone of lymph nodes, in the periarteriolar sheaths of spleen and in the thymus cortex. It was stated that the myxovirus neuraminidase cleaves the "homing" receptors of T cells for the lymphatic tissues.

Lymphopenia may be related to impaired proliferation or destruction of host Th cells. The majority of HIV-infected Th cells become chronic provirus carriers. Intermittent stimulation with other accidental infectious agents would activate HIV replication in a random selected carrier CD4 T cell. Productive replication of HIV in a few T cells would generate thousands and/or million virions released into the bloodstream and lymphatic tissues, infecting additional CD4 T cells and/or APCs equipped with alternative HIV receptors. In the case of viremia caused by the measles virus, the activated T lymphocytes and macrophages are productively infected and therefore "occupied" with the synthesis of virus-coded proteins and they loose their capacity for IL and/or cytokine synthesis. This event contributes to the state of transient virus-induced ISP elicited mainly by APCs involvement and impaired Th cell function. In contrast to infected macrophages, the adsorbed measles virions would not start productive replication in resting memory T lymphocytes, which have very few ribosomes. Productive replication of measles virus rather occurs in activated T cells proliferating in the presence of IL-2.

4. Reactivation of latent virus infection in the course of ISP

4.1. Human cytomegalovirus (HCMV)

HCMV referred to as the Human herpesvirus 5 (HHV-5) belonging to the subfamily *Betaherpesvirinae* has the most complex genome among herpesviruses. Its dsDNA encodes about 180 proteins. More than half of the virus-coded polypeptides are non-structural ones, having important functions including viral DNA replication (Mocarski and Curcel, 2001). A group of non-structural immediate early (IE) proteins, encoded by regions IEl and IE2, transactivates the transcription of early and late viral genes and probably plays a regulatory role in virus reactivation. Out of several IE transcripts, the translation of transactivator proteins IE1/72K and IE/86K is of highest importance (Spektor, 1996; Stenberg, 1996). To initiate efficient IE transcription at acute primoinfection, the virion tegument (located between the capsid and the envelope) carries the phosphoprotein pp71/UL82, which acts as co-factor for the cellular RNA polymerase II. In its absence (i.e. at reactivation of latent virus) the transcription of vDNA is slow, since it occurs solely as a result of the coordinated action of cellular transcription factors, such as activator protein 1 (AP1), signaling protein 1 (SP) and NFκB (Griffits and Emery, 2002).

At least 11 non-structural proteins interfere with the induction of immune response, namely with yielding of viral antigenic peptides recognized by TCR, with antigen processing, IFN induction and with the action of interleukins and chemokines (Table 2). The immune evasion proteins, which delay immune recognition and the slow generation of IE transactivator proteins in the absence of the transcription initiation factor pp71/UL82 favor a lasting balance between minimum virus production and its elimination. A good model for the understanding of this phenomenon is the guinea pig model of CMV latency. During acute infection the guinea CMV replicates in blood mononuclear cells, capillary endothelium cells, brain astrocytes, renal and salivary gland epithelium cells, placental trophoblasts of the pregnant females and

fibroblasts (Alford and Britt, 1990). The presence of minimal amounts of CMV produced in above mentioned tissues can be tested by adding indicator-cells sensitive to CMV replication. However, there may be difficult to distinguish between the non-productive and productive forms of CMV latency. Low amount of infectious virus can be produced in a few CMV DNA carrier cells and this state may be referred to as persistent infection. In fact, the non-productive latency is defined as the presence of vDNA in the absence of infectious virus production. Such a latency can reflect the presence of a few hundred copies of CMV DNA per ml of plasma (about one copy per 1 of 10^5 peripheral blood leukocytes). When the white blood cells from seropositive subjects are transferred to a seronegative recipient, the post-transfusion CMV syndrome develops in such subject (Diosi *et al.,* 1969; Weller, 1971). The seronegative recipient not only lacks protective antibodies but at the same time has no specific memory T and B cells at immediate disposal. Thus, such a recipient is not able to mount a quick immune response, so the reactivation of transferred latent CMV and its subsequent replication seem inevitable (Pass, 2001).

In mice infected with murine CMV, elimination of T lymphocytes at experimental ISP leads to activation and subsequent virus replication in peripheral blood white cells associated with extensive viremia. A similar effect was achieved by using antilymphocyte serum. This is consistent with importance of MHC-restricted Tc lymphocyte response for recovery from cytomegalic disease that develops in bone marrow transplant recipients (Quinnan *et al.,* 1982). Moreover, Th lymphocytes and IFN-γ production are essential at limiting the reactivation and dissemination of latent CMV (Hebart *et al.,* 1997). An important factor enhancing the low-grade reactivation of latent CMV is the stimulation of T cells by intercurrent infections. During ISP, the low level of virus production can be shifted to extensive virus production and manifested as reactivation. Iatrogenic ISP impairs the clonal proliferation of CMV-specific Th and B cells or the cooperation between APC and Th cells. The cytokine production is not reduced because it can be triggered in "bystander" cells within the lymph nodes. Activated T cells of heterologous specificity may not function "properly" in the specific anti-CMV defense. Their presence is undesirable since they can be permissive for virus production due to their stimulated state.

The most dramatic example of overwhelming CMV production following activation of previously established latency is the effect of accidental HIV infection. The probability of latent CMV activation as result of HIV-1 infection of a seropositive host is at least 10% and the expected reactivation rate increases by time (Francisci *et al.,* 1997). The lower is the level of Th lymphocytes in the course of AIDS, the higher is the probability of concomitant cytomegalic disease (Pass, 2001). At low levels of Th cells (100–200/ml) the probability of co-infection with reactivated CMV is 20%. The most frequently occurring cytomegalic disease in AIDS is chorioretinitis occurring in 80% of HIV-related complications, less frequent is colitis and/or esophagitis in 9% of cases. The interstitial pneumonia as well as hepatitis can develop in some patients (Cheung and Teich, 1999). It was postulated that CMV and HIV-1 potentiate each other (Barry *et al.,* 1990), since the CMV IE transactivator proteins act on the LTR sequence of HIV provirus enhancing the transcription of *Tat* protein. In turn, *Tat* increases the IE CMV transcription. Thus, in the presence of CMV replication the HIV-1 production enhances. Cytokines produced in response to CMV infection stimulate uninfected Th lymphocytes, which become targets for HIV replication (Ostrowski *et al.,* 1998). In dual congenital and postnatal infections with CMV and HIV-l, the frequency of severe clinical forms of cytomegalic diasease increases (Mussi-Pinhata *et al.,* 1998; Kovacs *et al.,* 1999).

Iatrogenic ISP enhances CMV reactivation following transplantation of organs, which carry the "endogenous" latent virus (Gardner *et al.,* 1974). The seronegative recipients are so sensitive to reactivation of latent CMV present in the donor tissues that it occurs with a probability of nearly 100%. Bone marrow and/or renal transplantations from seropositive donor to a seronegative recipient reflects the frequency of CMV latency in the donor (about 40–60% of domestic population is seropositive and consequently harbors the latent virus). When calculating with these prevalencies, then transplantation of bone marrow and/or kidney from any donor to a seropositive recipient would cause reactivation in about 20% of recipients (Metselar and Weinar, 1989; Wiesner *et al.,* 1993). This fact testifies a partial protection provided by specific memory Tc lymphocytes in the seropositive recipient. Nevertheless, testing of antibodies by ELISA is simple and provides relevant information. The development of CMV disease is less frequent, when the kidney or bone marrow was transplanted from a seronegative donor to seropositive recipient (Ljungman, 1996). In such patient, ISP therapy and rarely post-operation stress may reactivate the latent CMV harbored in the recipient. The chance of successful ganciclovir therapy is high, especially when the ISP therapy can be interrupted. Summing up, absolutely safe from the viewpoint of CMV complications is organ transplantation from a seronegative donor to a seronegative recipient. This transplantation is associated with a low risk of post-transplantion cytomegalic disease. The transplantation from a seropositive donor to a seropositive recipient is possible provided that careful monitoring of CMV viremia will follow (Einsele *et al.,* 1995). The transplantion of any organ from a CMV seropositive donor to a CMV seronegative recipient was prohibited in many countries.

The most frequent post-transplantation complication, which occurs in seropositive recipients after renal or bone marrow transplantations, is interstitial cytomegalic pneumonitis (Meyers *et al.,* 1982), less frequently hepatitis, enterocolitis and/or chorioretinitis. Increased viremia, viruria and CMV excretion in saliva may occur as silent complications. The presence of CMV DNA in the stool accompanies viral colitis and its presence in the sputum is associated with pneumonitis. In pneumonia, the number CMV DNA copies/ml blood is very high (more than 10^6 CMV DNA copies/ml) as compared with the low copy number reflecting latency. A good indicator of acute viremia is the CMV antigenemia, namely the tegument protein pp65 detected by ELISA (van den Berg *et al.,* 1989; Boland *et al.,* 1990). In addition, this protein can be detected in smears or extracts of leukocytes (Gerna *et al.,* 1991; Boeckh *et al.,* 1992; Grefte *et al.,* 1992). The rapid culture method using immunofluorescence (IF) was recommended in indicator cells infected with urine sediments containing CMV (Gleaves *et al.,* 1984). The testing of antigenemia is still less sensitive than detection of CMV DNA by PCR (Boland *et al.,* 1992). The prophylaxis with ganciclovir or valganciclovir is recommended in any bone marrow transplant recipient with critical levels of total blood neutrophil counts and in those who develop viremia exceeding the minimum threshold indicating CMV persistence. In severe cases of pneumonia, the intravenous ganciclovir therapy should be combined with interruption of ISP therapy. In the case of combined HIV and CMV infections, treatment with foscarnet or cidofovir (non-selective DNA polymerase inhibitors) and formivirsen (antisense IE CMV mRNA inhibitor) is recommended (Inouye *et al.,* 2002).

4.2. Epstein-Barr virus (EBV)

The EBV also referred to as Human herpesvirus 4 (HHV-4) can establish three forms of latency. Latency type I is characterized by expression of a single latency-associated protein designated EBNA-1 and occurs in carrier B lymphocytes and in nasopharyngeal epithelium cells (Rickinson and Kieff, 2001). At primoinfection, often manifested as acute infectious mononucleosis (IM), the B lymphocytes express several latency associated proteins; this state is referred to as type II and/or III latency. In latency type III the B cells express at least 2 latency-associated proteins causing false intracellular signaling (Table 4). Therefore, they get immortalized (Sugden, 1994). The crucial protein causig immortalization of non-productively infected B cells is the latent membrane protein 1 (LMP-1). B cells expressing LMP-1 and/or EBNA-2 are eliminated from the circulation by the help of antigen-specific Tc lymphocytes. Cytotoxic T cells recognizing B lymphocytes of the latency types II/III are enlarged to form atypical mononuclear cells – the hallmark of IM. In contrast to B cells, the complete reproduction of EBV occurs in the nasopharyngeal epithelium cells EBV. The virus-coded IL-10 facilitates its replication in nasopharyngeal mucosa (Beauliou and Sullivan, 2002).

In the immunocompromised host with HIV infection, iatrogenic ISP or X-chromosome linked immunodeficiency the IM syndrome has a severe – often lethal – course (Sullivan and Woda, 1989; Craighead, 2000a). During EBV reactivation of such host, lymphoproliferative disorders develop, such as hairy leukoplakia (HLC), post-transplantation lymphomas (PTL) and/or AIDS-associated lymphomas. The X-chromosome linked lymphoproliferative

Table 4. Latency associated genes expressed in EBV infected B lymphocytes

EBNA-1 is a dsDNA-binding protein, which recognizes a typical sequence within the Ori_p region and regulates the replication of the episomal plasmid-like EBV DNA. The EBNA-1 in present in the nuclei of B lymphocytes during latency I.
EBV early RNA is non-polyadenylated RNAs (EBERs) do not serve translation. They associate with PKR, an IFN-I induced cellular enzyme; this complex reverses the IFN action at ribosomes. EBERs together with EBNA-1 are the hallmark of latency I.
EBNA-2 is an important transactivator protein, which induces expression of many B cell marker proteins, such as CD21 and CD23. It also activates he expression of LMP-1.
EB nuclear antigen leader protein (EBNA-LP) regulates the expression of EBNA-2 and LMP-1.
LMP-1 is the main protein related to immortalization of the EBV DNA. It is a transmembrane polypeptide, which outer domain has a portion analogous to TNFR, while the cytoplasmic domain binds several adaptor proteins, such as the TNF receptor coupled intracellular factor (TRAF) as well as cellular vimentin. As a source of false positive signaling, LMP-1 activates the translocation of NFκB to the nucleus, leading to expression of EBNA-2 and vIL-10. LMP-1 has an antigenic domain recognized by T cells.
Transactivator protein *Zta* is translated from a transcript that transcription is governed by the Cp/Wp promoter. This protein is not regularly transcribed during latency III, it is a co-factor initiating early transcription. About 30 proteins are synthesized, including antigens EA-R and EA-D. A group of early proteins participates in the synthesis of viral DNA, forming the DNA polymerase complex.

disease (Duncan's disease) is a peracute form of IM occurring only in male children. This disease is characterized by the explosive proliferation of B cells and T cells-derived atypical mononuclear cells, which permeate the enlarged lymph nodes, spleen and liver, causing multiple functional defects, tissue necrosis and apoptosis in the thymus (Tazawa *et al.,* 1996). There may be inherited brakes on the chromosomal band Xq25 and consequently the appropriate Th2 type cytokine response is impaired (Seemayer *et al.,* 1993). The major defect is the lack of the signal-associated polypeptide otherwise present in T lymphocytes and NK cells. Signal-associated polypeptide is an adapter protein for the CD150 receptor involved in Th and B cell interactions (Sayos *et al.,* 1998; Fischer and Thornley-Lawson, 1991). Taken together, in congenital fulminant IM the intracellular signaling within Tc and NK cells completely fails despite of their stimulation by LMP-1 expressed on the surface of B cells. The reason for the severe course of IM is that the B cells in the latency of type III proliferate in uncontrolled manner.

In immunocompromized patients positive for EBNA-1 antibodies, two different syndromes may occur. HLC is a mild disease in consequence of EBV replication in the mouth cavity cells during AIDS (Greenspan *et al.,* 1984). A similar syndrome may accompany iatrogenic ISP following bone marrow transplantation (Epstein *et al.,* 1988). Hairy projections are the site of acanthosis and parakeratosis containing enlarged balloon-like cells revealing intranuclear inclusions. The EBV virions are visible by electron microscopy (Ferbas *et al.,* 1992). The production of EBV virions within the oral cavity epithelium cells is also the sign of reactivation of latency type I due to intercurrent HIV-l infection or medical ISP. The EBV-negative recipient may be infected with EBV, but more probably latency type I carrier B lymphocytes coming from the donor reactivate (Walker *et al.,* 1995; Hopwood and Crawford, 2000).

The post-transplantation lymphoproliferative disease (PTLD) is based on immortalization of the B lymphocytes (Hanto *et al.,* 1984; Basgoz *et al.,* 1995). PTLD develops mainly following heart transplantation, but may occur also in association with kidney, bone marrow and liver transplantations. Lymphoma cells containing proliferating B cells occasionally mixed with T cells infiltrate the mediastinal as well as other lymph nodes. They also may infiltrate the graft tissue causing its rejection (Nalesnik *et al.,* 1988). Such uncontrolled proliferation of immortalized B cells expressing the transforming LMP-1 and EBNA-2 proteins takes place due to impaired Tc lymphocyte response in the ISP treated recipient. However, the pathogenesis of various forms of PTLD is even more complicated. Several categories of this disorder were recognized: a) monoclonal but polymorphic B cell hyperplasia or lymphoma lacking cell division regulator p53 abnormalities; b) monoclonal immunoblastic lymphoma or myeloma with alterations in the p53, *N-ras* and c-*myc* oncogenes; c) polyclonal and polymorphic multiple plasmacytic hyperplasia with no alterations in regulatory oncogenes (Knowles *et al.,* 1995). To prevent rejection, infusion of syngenic Tc lymphocytes from an EBNA-1 antibody positive donor was recomended (Heslop and Rooney *et al.,* 1997).

An interesting animal model for studying EBV-related lymphoproliferations is the infection with the Murid herpesvirus 4 (MuHV-4) in mice (Rajčáni and Kúdelová, 2005). MuHV-4-infected mice develop an acute IM-like syndrome as well as chronic lymphoproliferative disease, which may be occasionally manifested with a leukemia-like course. The probabilily of lymphomas in chronic MuHV-4 infections may be as high as 11% (Mistríková *et al.,* 1999).

4.3. Varicella-zoster virus (VZV)

VZV referred to as Human herpesvirus 3 (HHV-3) belongs to the subfamily *Alphaherpesvirinae.* The VZV DNA encodes about 70 non-structural and structural proteins, fairly less than the HCMV (Cohen and Straus, 2001). In the host, VZV spreads along nerves (axonal spread), but also via blood stream (Arvin, 2001). During early phases of acute infection, VZV replicates in blood leukocytes especially in T lymphocytes (Ozaki *et al.,* 1986; Asano *et al.,* 1990; Sawyer *et al.,* 1992). It is not quite clear whether productive replication occurs in B lymphocytes and macrophages (Mainka *et al.,* 1998). The proportion of blood leukocytes containing VZV-specific antigens is not very high and ranges between 0.001% to 0.01% (i.e. <l:10,000 leukocytes of the total buffy coat population) (Koropchak *et al.,* 1991). The early phase viremia occurring during the 10-days incubation period is a consequence of initial VZV replication in nasopharyngeal epithelium cells, which serve as the portal of entry. Secondary viremia develops as result of virus replication in the regional cervicofacial lymph nodes. During the secondary viremia VZV reaches the skin and typical pustular vesicles are formed in consequence of virus replication in epidermal squamous epithelium cells. Here VZV enters the free nerve endings and spreads along nerves to the neurons and satellite cells of regional sensory ganglia (Gershon and Silvertein, 2002).

It was noticed many years ago that in children with hypo- or gammaglobulinemia the course of acute varicella is not more severe as compared to children with physiological gammaglobulin levels. However, the course of varicella is very severe in children with innate alterations of cell-mediated immunity and in other immunocompromized children, i.e. in congenital HIV infection (Jura *et al.,* 1989; Derryck *et al.,* 1998), in children treated with corticosteroids and in those suffering of leukemia or Hodgkin's disease (Feldman and Lott, 1987; Gershon *et al.,* 1997). In these

children, the pustules are large and hemorrhagic and viremia is high especially if associated with pneumonitis. Shock and intravascular coagulation are frequent complications related to the capillary endothelium damage and formation of vasoactive substances in combination with inflammatory chemokines (Singh and Deng, 1998). Hemorrhagic changes of skin pustules develop due to thrombocytopenia. As mentioned, the virus which enters nerve endings spreads to thoracic and lumbosacral sensory ganglia (Raggozino *et al.,* 1982). In the immunocompromized host or in elderly persons latent VZV reactivates causing recurrent herpes zoster. The zosteriform vesicles are situated in the dermatome of sensory ganglia in which the latency had been established. Healing of the herpes zoster vesicles depends of local IFN-I production, mobilization of virus-specific memory T lymphocytes in local lymph nodes and their proliferation due to cytokine production. The TCR of Th and Tc lymphocytes recognize the virus-specific antigens synthesized de novo in infected skin epidermal cells. Certain non-structural antigens are recognized by Th cells, processed in dendritic cells and presented via their MHC-I molecules (cross presentation). The structural envelope glycoproteins as well as some non-structural proteins in the infected cell membranes become targets for lysis by NK cells and by VZV-specific Tc lymphocytes (Arvin *et al.,* 1986; Arvin, 1996).

The process of mobilization of specific Tc lymphocytes may be slow and less efficient in elderly persons. Therefore, herpes zoster occurs more often in elderly persons than in young VZV carriers. The frequency of virus-specific T lymphocytes decreases by age below the threshold of a single specific cell per 10^5 T cells and this fact may contribute to higher frequency of herpes zoster in the elderly patients. Since satellite cells of infected regional sensory ganglia may continuously produce low amounts of VZV either in the pre-eruption as well as in post-eruption periods, the impaired immune T cell control is not the only cause of the higher incidence of clinical signs, but can also explain the mechanism of post-herpetic neuralgia. This hypothesis was confirmed by decreased skin reactivity to VZV antigen by delayed hypersensitivity test in the elderly and/or immunocompromized subjects (Kamiya *et al.,* 1977).

Herpes zoster often develops due to severe intercurrent disease, i.e. in tumors, leukemia, during iatrogenic ISP, in inherited and/or acquired immunodeficiency, such as AIDS (Baba *et al.,* 1986; Buchbinder *et al.,* 1992; Glesby *et al.,* 1995). The healing of herpes zoster lesions is impaired in the case of ISP and post-herpetic long-lasting neuralgia is common (Watson and Evans, 1986). As already mentioned, the persistent infection of satellite cells within the ganglion is a frequent feature of delayed healing of herpes zoster related to ISP. Liquor may also contain low amount of infectious virus that is difficult to isolate. Nevertheless, VZV DNA can be more easily detected by PCR (Haanpaa *et al.,* 1998). Meningoencephalitis occurs due to VZV, which has reactivated within the ganglia, and appears in the course of AIDS (Gilden *et al.,* 1988). Ateritis of brain vessels may occur in elderly patients suffering of exhausting intercurrent disease (Kleinschmidt-deMasters *et al.,* 1996). During the ISP treatment of VZV-positive persons, more severe forms of herpes zoster occur in comparison to patients without ISP treatment. The healing of vesicular lesions in patients with ISP treatment is delayed and the frequency of post-herpetic complications is high. The extent and number of vesicular lesions in patients with tumors is usually greater (Feldman *et al.,* 1977). In AIDS patients, reactivation of VZV is associated with tongue hyperkeratosis lesions similar to those caused by EBV (Leibovitz *et al.,* 1992).

4.4. Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2)

HSV-1 and HSV-2 also referred to as Human herpesvirus 1 and 2 (HHV-1, HHV-2) are the most thoroughly studied human herpesviruses (Rajčáni and Ďurmanová, 2001; Roizman and Knipe, 2001; Whitley, 2001). The HSV-1 genome codes for 84 proteins and at least 7 ORFs are doubled and 3 or possibly 4 gene pairs occupy the opposite DNA strand (Rajčáni *et al.,* 2004). The viral envelope has not less than 11 glycoproteins, which are involved in virus adsorption and penetration. The tegument is located in between the capsid and the envelope and contains the transcription initiation factor VP16/α-TIF (Hayward, 1993). Both HSVs spread along axons mainly by fast axonal transport (Johnson and Mims, 1968; Hill *et al.,* 1972; Rajčáni *et al.,* 1979). Usually, the latency of HSV-1 is established within the Gasserian ganglion of trigeminal nerve. While in the VZV latency, at least one IE protein is expressed, in the non-productive HSV latency only latency associated transcripts (LAT) are made. The LATs are anti-sense molecules, which do not show the structure of mRNA (Gordon *et al.,* 1988; Spivack and Fraser, 1988). Their function is not quite clear, but they are involved in latency activation rather than in its establishment (Perng *et al.,* 1994; Rajčáni and Ďurmanová, 2000). The mechanism of LAT action is complex; several authors believe that LATs may inhibit the formation of IE HSV mRNAs especially by interfering with the transcription of the IE/ICP0 mRNA (Mador *et al.,* 1998; Preston, 2000; Nsiah *et al.,* 2001).

At the end of early post-infection phase, the class IgM antibodies are produced along with the induction of the specific Th lymphocyte response. At later intervals of primoinfection (days 6–7 since the onset of herpetic stomatitis), class IgG antibodies prevail along with the specific Tc lymphocyte response. At this stage of primoinfection there is already difficult to isolate infectious virus that was abundantly produced in the early primo-

infection phase. The latency is regularly established in the course of primoinfection, regardless whether silent, abortive or showing typical signs of disease. During latency that lasts probably lifelong, the IgG antibodies may be present for a long period. Although their level decreases by time, spontaneous recurrencies (either clinically manifested of silent reactivations) repeatedly increase the IgG titer. Memory Tc lymphocytes surviving in the regional lymph nodes proliferate in association with any reactivation of the latent virus. The relatively frequent appearance of virus production in a few neurons of sensory ganglion attracts virus-specific Tc lymphocytes (Nash, 2000). According to recent investigations, either the IE transactivator protein ICP4 and/or the gB precursor polypeptide (produced at early-late kinetics) reveal antigenic domains by virus-specific Tc cells (Wallace *et al.,* 1999; Khanna *et al.,* 2004). The prompt cytotoxic T cell response in association with the synthesis of a few HSV-coded polypeptides may suppress the transition to productive virus replication. The delicate balance between abortive reactivation and production of small amounts of infectious virus seems to be under immune control. Kočišová *et al.* (1988) described inapparent episodes of HSV-1 shedding in the tears of rabbits with established latent infection. During a 211 days follow-up, spontaneous virus shedding was observed in 47% of the rabbits; the ISP regimen increased the rate as well as the duration of reactivation periods by approx. four times.

The recurrent orofacial blisters (herpes labialis) resulting from spontaneous reactivation occurs in 16–32% of total population at least once a year. The HSV-1 antibody prevalence ranges in USA from 40 to 60% (Douglas *et al.,* 1970; Ship *et al.,* 1977) and in the Slovak republic the antibody prevalence reaches 80% (unpublished observations) and in other countries nearly 90%. The reactivated virus spreads by retrograde axonal transport to the skin and facilitates a development of clinical forms of reactivation, especially the recurrent blister formation. The increased levels of prostaglandin E favor the replication of HSV-1 (Hill *et al.,* 1976) and may explain the effect of UV-irradiation during solar dermatitis. The extent of HSV replication in the skin dramatically increases in case of eczema and atopic dermatitis, when widespread dermatitis develops along with secondary viremia (Wheeler and Abele, 1966). Primo-infection might have a severe course and show prolonged healing in patients with eczema (Terézhalmy *et al.,* 1979).

Various local influences, such as mechanic stimulation of virus inoculation site, chemical stimulation, surgery on the trigeminal nerve etc., would provoke reactivation. Following bone marrow, liver, kidney and heart transplantations and during exhausting malignant disease, the latent HSV-1 would reactivate along with CMV (Muller *et al.,* 1972; Pass *et al.,* 1978). The mobilization of memory Tc lymphocytes is efficient only in healthy subjects. However, under conditions of ISP, the healing of recurrent herpetic blisters is impaired. Minute amounts of HSV produced during spontaneous reactivation, would cause widespread skin lesions as well as viremic complications during ISP treatment. In immunocompromized patients, HSV spreads on the skin infecting small wounds occurring at shaving or other negligible injuries. The virus spreads from the mouth cavity to the squamous epithelium of oesophagus or to upper respiratory pathways (Whitley, 2001). The extent of such complications is directly related to the duration of ISP treatment (Rand *et al.,* 1977). The virus does not remain restricted to neural pathways and viremia occurs. In experimental ISP, the lethality of HSV-1 infection increased at least 10 times (Rajčáni *et al.,* 1974). The reactivated virus can spread to the external and internal surfaces covered by squamous epithelium, to the connecting mucosa covered by cylindric epithelium (ano-sigmoidal, gastric and tracheal involvement) and via blood to kidneys, adrenal glands and pancreas (Safrin *et al.,* 1991). Especially dangerous is the dissemination of HSV strains resistant to acyclovir therapy. In HIV infections, HSV-2 may frequently reactivate causing virus shedding from genital area (Schacker *et al.,* 1998; Wald *et al.,* 2000).

4.5. Kaposi's sarcoma-associated herpesvirus (KSHV)

The recently discovered gammaherpesvirus also referred to as Human herpesvirus 8 (HHV-8) has been grouped into genus *Rhadinovirus.* The KSHV virions are produced rarely and can be seen within elongated fibroblast-like sarcoma cells forming the malignant KS associated with AIDS (Friedman-Kien, 1981; Walter *et al.,* 1984). Mature capsids and complete enveloped virions are frequently found in cell lines derived from primary effusion lymphoma (PEL), which is body cavity lymphoma developing during AIDS (Gaidano *et al.,* 1996; Hsi *et al.,* 1998). In HIV-negative subjects, the benign lymphoid proliferation was originally referred to as pyothorax associated – non-Hodgkin – lymphomas (PAL). These lymphomas do not contain KSHV DNA, but can be EBV positive. PAL develops in chronic pleural infections or in tuberculosis pleuritis (Carbone *et al.,* 1996; Cesarman *et al.,* 1996; Hermine *et al.,* 1996).

The classical form of KS mainly occurs in the elderly man from Mediterranean basin (Craighead, 2000c). Without involving internal organs, the affected skin areas are infiltrated by slowly growing tumor-like fibroblasts and chronic inflammatory cells. The more aggressive form of KS referred to as endemic KS occurs in young adults with latent KSHV infection in Middle Africa, where the KSHV spreads per saliva and by sexual contact (Olssen *et al.,* 1998; Gessain *et al.,* 1999), but has no relationship to AIDS. The endemic KS shows invasive growth and metastasis to lymph nodes, respiratory and gastrointestinal tracts in ISP subjects

Table 5. EBV and KSHV proteins associated with cell division and immortalization

EBV gene	KSHV gene	Protein (function)
BNLF1	K1	LMP-1, latent membrane protein 1 (EBV/LMP-1 and KSHV/K1: both activate intracellular signaling via different mechanisms)
LMP-2	K15	LMP-2, latent membrane protein 2 (tyrosine kinase which associates with LMP-1)
BALF1	ORF16	*vBcl*-2, viral B cell leukemia 2, anti-apoptotic protein, viral analog of cellular *Bcl-2*
BKRF1	ORF72*	vCyclin, cellular cyclin D analog
none	ORF71*	vFLIP, *Fas*-associated death domain like inhibitory protein, an ICE (IL-1β converting enzyme and/or caspase 1) inhibitor, anti-apoptosis protein
none	K12	Kaposin, a latency associated transformation protein, prolongs the activated state of the growth factor receptor upon external stimulation, stabilizes the cytokine mRNAs for KSHV proteins, activates MK2 (mitogen kinase 2)
BLR1	ORF74*	GPCR (G-protein coupled receptor, IL-8R homolog) increases the sensibility to cytokine signaling promoting B cell and/or KS cell proliferation
EBNA-2	ORF73*	EBNA-2, LANA-1 (latent nuclear antigen 1 of KSHV and/or the EB nuclear antigen 2) promote cellular DNA synthesis

*Tandem regulation of transcription was described.

(Akhtar, 1984), especially after renal transplantation. The invasive form of KS in HIV-negative patients with previously established KSHV latency is referred to as iatrogenic KS (Qunibi *et al.,* 1988). Taken together, there are two non-invasive (classical and endemic) as well as two invasive forms of KS. The latter occur either in HIV-positive or HIV-negative subjects who underwent iatrogenic ISP. In AIDS, the KSHV DNA-specific fragments were identified within elongated KS cells by a special technique called subtractive polymerase chain reaction (Lysitsin *et al.,* 1993; Chang *et al.,* 1994). Later on, the productive replication of KSHV was detected in cells derived from PEL or from KSHV-associated lymphomas also called angiofollicular lymphoid hyperplasia.

The pathogenesis of KS is very complex. The KSHV latency can be established in B lymphocytes, CD68 mononuclear phagocytes, capillary endothelium cells, and occasionally in glandular epithelium cells of prostata (Whitbey *et al.,* 1995; Staskus *et al.,* 1997). Reactivation of the virus is a stepwise process with a long period of partial expression of latency associated and early non-structural proteins; some of the latter cause transformation of host cells (Table 5), others are cytokine and/or chemokine analogs modulating the immune response (Table 2). KSHV encodes the macrophage inflammatory proteins vMIP1 and vMIP2, the viral interferon regulation factor K9/vIRF, the viral IL-6/K2 and the viral *Fas*-associated inhibitory protein vFLIP/K13. The vIL-6 causes B cell proliferation and indirectly promotes the proliferation of endothelium cells, when inducing vascular endothelium growth factor. The abundant vIL-6 production in B lymphocytes can be induced by the HIV-1 transactivator protein *Tat*, which also activates early transcription of CMV genes (Ensoli *et al.,* 1993, 2000). The hybrid molecule vFLIP is a caspase inhibitor (IL-1β converting enzyme inhibitor) activated by apoptotic stimuli. Important for the KS cell proliferation is the KSHV-coded *vBcl-2*, which has a domain homologous with the cellular *Bcl-2* anti-apoptotic protein (Rajčáni and Kúdelová, 2003). In addition, KSHV encodes a viral cyclin (Chang *et al.,* 1996; Kennedy *et al.,* 1999), a cellular cyclin D analog. This interacts with *Cdk6* (cyclin dependent kinase 6) pushing the cell growth cycle from phase G1 into phase S (Swanton *et al.,* 1997). Interestingly, the viral cyclin cannot be downregulated by the cellular mitosis inhibitor proteins belonging to the KIP (kinase inhibitor protein) system. In opposition, the p27/KIP is degraded by the vCyclin/cdk6 complex (Ellis *et al.,* 1999). Finally, the KSHV encodes a latency associated nuclear antigen (LANA-1), which cooperates with viral DNA synthesis and binds the anti-onc protein p53 (Friborg *et al.,* 1999). Elimination of the cell division regulator p53 makes LANA-1 a leading oncoprotein candidate, which lifts the cell cycle arrest and removes the apoptosis blockade (Garber *et al.,* 2002).

The most frequent post-transplantation complication occurring due to reactivation of KSHV latency is the development of mediastinal non-Hodgkin B cell lymphoma following heart transplantation (Dotti *et al.,* 2001). This tumor originally referred to as body cavity based lymphoma (BCBL) was later designated as PEL. This disease consists of anaplastic, intensively growing lymphoid cells with nuclei that are LANA-l positive. PEL cells contain KSHV DNA and may express several other non-structural KSHV proteins. When propagated *in vitro*, they would produce herpesvirus capsids upon chemical induction. It is believed that the

benign angiofollicular hyperplasia (Castleman disease) is related to the KSHV coded vIL-6 activity (Parravicini *et al.,* 1997).

In contrast to the testing of CMV antibody in patients before transplantation, detection of anti-KSHV antibodies is not a routine pre-transplantation procedure. The smears from PEL-derived cells are used as antigen for IF (Miller *et al.,* 1997) or soluble antigen extracts were prepared from KSHV producer cells for ELISA. Recently, the recombinant capsid protein (ORF65) and envelope glycoprotein K8.1 were used as antigens for testing of the immune response. The detection of LANA-1 antibodies is a frequently recommended test. Due to difficulties with the KSHV and in accord with the low positive rate of KSHV DNA distribution (about 5% in our region), there is no reason to insist on KSHV serology in the pre-transplantation diagnostic. However, in the Mediterranean basin countries, the prevalence of KSHV may be higher (8–10%). In North and Middle African countries, where the prevalence may reach even 50–70% (Moore and Chang, 2002), there is reasonable to test KSHV antibodies of the donor and recipient especially in the case of heart transplantation.

4.6. Human herpesviruses 6 and 7 (HHV-6, HHV-7)

Two recently discovered human herpesviruses belong to the subfamily *Betaherpesvirinae,* genus *Roseolovirus*. HHV-6 is a lymphotropic virus, which infects T lymphocytes (Salahuddin, *et al.,* 1986; Ablasi *et al.,* 1987; Frenkel *et al.,* 1990). Acute primoinfection with the HHV-6 subtype B causes syndrome of fever with skin rash called roseola or *exanthem subitum*. HHV-6 subtype B latency can be established at a relatively low incidence rate in peripheral mononuclear cells of healthy adults (Kondo *et al.,* 1990; Rajčáni *et al.,* 1994). Reactivation of HHV-6 is manifested by a fever and skin eruption; it may occur following renal and kidney transplantation (Singh *et al.,* 1995, 1997). Leukopenia, trompocytopenia and allograft rejection may develop along with interstitial pneumonia and mild encephalitis in association with transplantation (Okuno *et al.,* 1990; Drobyski *et al.,* 1994). The reactivation of HHV-6 has a less severe course. Usually asymptomatic viremia accompanied with fever develops as the result of production of IL-1β and TNF (Cone *et al.,* 1999). HHV-6 replication increases the expression of CD4 also on CD8 cytotoxic lymphocytes, which enhances the opportunity of HIV-1 infection (Lusso *et al.,* 1991).

The reactivated HHV-6 acts on the U3 region of the long terminal repeat (LTR) of integrated HIV-1 provirus, increasing the probability of activation of latent HIV infection (Pellett and Dominguez, 2001). This function was mapped at the putative immediate early region of the HHV-6 genome (Martin *et al.,* 1991). Reactivation of HHV-6 in combination with HIV-1 and CMV replication causes brain damage, although these three viruses replicate in different brain cells. The reactivation of HHV-7 is most frequently associated with CMV reactivation what increases the danger of graft rejection already in the absence of cytomegalic disease (Kidd *et al.,* 2000). Since immune reactivity varies with the age of individual, various syndromes linked to HHV-6 reactivation are atypical polyclonal lymphoproliferation, heterophil antibody negative IM-like syndrome, necrotizing lymphadenitis (Kikuchi's disease) and malignant lymphomas (Horwitz *et al.,* 1992; Krueger *et al.,* 1992; Kikuchi *et al.,* 1992).

4.7. Human polyomaviruses

Polyomaviruses are small DNA viruses 45 nm in diameter, which infect mainly rodents and lagomorphs but also humans. The naked polyomavirus capsid consists of 72 capsomers and contains a circular dsDNA molecule approximately of 5000 bp length. The early region of polyoma DNA encodes 3 non-structural proteins termed T-antigen. The large T-antigen initiates replication of vDNA and regulates its transcription. The late region of vDNA encodes structural proteins (VP1, VP2 and VP3) and a non-structural protein called *agno*. In latency the BK virus (BKV) as well as JC virus (JCV) DNA persists in renal tubular epithelium cells. Immunocompromized subjects but accidentally also normal individuals may excrete polyomavirus in urine (Greenlay and O'Neil, 2002). In rodent cells, virus persistence with T antigen production is associated with stimulation of host cell DNA synthesis. JCV transforms rat fibroblasts and hamster glial cells (Frisque *et al.,* 1980; Major, 2001) and the polyomavirus DNA integrates into host cell DNA. In human cells that show long-term T- antigen expression, chromosomal damage may occur (Barbanti-Brodano *et al.,* 1998; Theile *et al.,* 1990).

Seroconversion for BK or JC viruses occurs in approximately 10% of children at the age of 5–7 years, later on in adults it may reach up to 76% (Taguchi *et al.,* 1982). At acute primoinfection, which usually has inapparent course, the virus enters the human body via respiratory tract and oral cavity. Fever occurs due to acute BKV infection, when associated with signs of upper or lower respiratory tract disease (Goudsmit *et al.,* 1982). BKV and JCV replicate in the nasopharynx and is excreted by saliva. Up till now, no systemic BKV infection has been reported in immunologically normal subjects (Reploeg *et al.,* 2001). The JCV DNA is found in B lymhocytes (Gallia *et al.,* 1997). In immunocompromized host, abundant quantities of JCV DNA could be found in B cells, especially in bone marrow and spleen. JCV-positive B lymphocytes were identified in perivascular infiltrates of the brain (Mori *et al.,* 1992; Ferrante *et al.,* 1995). In healthy persons showing no signs

of JCV-related brain disease, DNA amplification studies showed minimal amounts of JCV DNA in the brain extracts (White *et al.,* 1992). These findings suggest that latent JCV might persist in the brain for long time pointing at the source of reactivated virus in progressive multifocal encephalopathy (PML). For keeping the JCV latent in the extraneural tissues by elimination of productively infected cells, virus-specific cytotoxic T lymphocytes are of essential importance. Nevertheless, reactivated "endogenic" polyomaviruses are shed in saliva and urine from otherwise healthy subjects. The frequency of silent shedding increases under ISP conditions. Longitudinal studies in HIV-infected patients have documented the urinary excretion of BKV or JCV at a frequency of 37% or 22%, respectively (Knowles *et al.,* 1999).

Although our knowledge on the distribution of BKV and JCV in healthy adults is limited, both viruses remain latent in kidney tubular epithelium cells and possibly also at the portal of entry (nasopharyngeal tissues). During acute phase, JCV (less frequently BKV) spread throughout the body mainly via B lymhocytes, which may be positive for viral DNA and may occasionally express T antigen. The main feature of reactivation of the JCV is a putative, yet poorly defined Tc lymphocyte defect, which accounts for rare cases of PML developing in HIV-negative subjects as well as in the absence of any iatrogenic ISP regimen. The JCV isolates from such "idiopathic" cases of PML revealed mutations in the regulatory region of the genome allowing increased transcription of structural polypeptides (Agostini *et al.,* 1977). These forms of PML have to be distinguished from PML cases that develop in association with AIDS. The PML syndrome in AIDS does not develop as frequently as the CMV or KSHV infection.

Several non-AIDS cases of PML were described under conditions of severe exhaustive diseases, such as chronic tuberculosis, chronic leukemia and Hodgkin's disease. The classical PML is characterized as an inflammatory process in the white matter of brain hemispheres and/or in brain stem. Clinically, the disease is associated with the loss of sensory and motor skills, visual abnormalities, difficulties in phonation and swallowing, and corresponding muscular weakness. In addition, personality and intellect alte ations cannot be excluded (Richardson, 1961). Histology of the brain shows enlarged oligodendroglial cells with intranuclear inclusions, which contain polyomavirus capsids filled with JCV DNA visible by electron microscopy (zuRhein and Chou, 1965; Craighead, 2000d). In addition, astrocytes are semipermissive to JCV and may express T antigen (Greenley and Kineey, 1986) that binds cellular p53 favoring proliferation and formation of binuclear cells. Occasionally, limited production of virions occurs (Ariza *et al.,* 1994). The main feature determining the clinical symptomatology is demyelinization, which involves extensive areas of the white matter. PCR examination detects the presence of JCV DNA in the cerebrospinal fluid (Fedele *et al.,* 1999).

BKV has been consistently associated with interstitial nephritis, chronic haemorhagic cystitis and less frequently with interstitial pneumonia occurring in kidney and/or bone marrow transplantation recipients (Arthur *et al.,* 1986; Apperley *et al.,* 1987; Arthur and Shah, 1987). Such patients develop viruria and viremia. Theoretically, the reactivated BKV may come from the donor, but the possibility of reactivation of latency residing within recipient's tissues cannot be excluded. ELISA is used for determination of anti-BKV antibodies using a recombinant VP1 capsid antigen or other virus-specific polypeptides (Frye *et al.,* 1997; Hamilton *et al.,* 2000). In the cases of BKV-negative recipients, IgM and IgA of BKV-specific antibodies develop following bone marrow and/or renal transplantation, which do not cross-react with JCV antibodies and *vice versa* (Drumond *et al.,* 1987).

4.8. Human adenoviruses

Adenoviruses can be frequently isolated from humans and many animal species. They are naked particles of 70–100 nm in diameter that consists of 252 subunits. The 12 spikes of the icosaedral particle are accupied by the base penton protein (III), from which a fiber protein (IV) projects; the latter is responsible for hemagglutination. The purified penton protein is toxic for host cells. The most abundant capsid protein is the trimeric hexon polypeptide (II) with 3 additional proteins associated. The internal capsid contains dsDNA and 4 core proteins. Thus, the adenovirus genome organized into an early and a late region, codes for at least 11 structural and 21–22 non-structural proteins (Shenk, 2001).

At primoinfection, adenoviruses replicate in the oropharynx, tonsils and adenoids, which represent the main virus reservoir (Horwitz, 2001a). Most likely adenovirus replication occurs in non-ciliated respiratory epithelium cells, in the conjuctival epithelium, in the transient epithelium cells of urinary bladder and in the mucosa of lower gastrointestinal tract. B-lymphocytes and macrophages disseminate the virus during viremia. In experimental rat and mouse infection, the productive virus replication is found in the respiratory epithelium cells. The inflammation is related to Th l cytokine production (TNF) as well as to IL-1 and IL-6 induction. At the late post-infection intervals, the role of cytotoxic T lymphocytes predominates. Tc cells form the round cell infiltrate in adenovirus peribronchitis and/or interstitial pneumonia (Ruuskanen *et al.,* 2002). As shown in Table 3, adenovirus may evade the immune response by means of the early E3 protein, which inhibits TNF; another E3 coded polypeptide inhibits the transport of MHC-I components (Mahr and Gooding, 1999; Horwitz, 2001b).

Adenoviruses persist in adenoid vegetations of nasopharynx and in tonsils (Hillis *et al.,* 1973). The carrier B lymphocytes may harbor the viral DNA in a non-productive state (Horvath, 1986; Neumann *et al.,* 1987).

The persistence of adenovirus DNA explains the virus reactivation under ISP conditions. Following the bone marrow transplantation, reactivated virus may come from the donor as well as from the recipient (Harnett *et al.,* 1982). Adenovirus excretion starts in urine and/or stool. A proportion of transplantation recipients develop hemorrhagic cystitis, interstitial pneumonia and gastroenteritis (Flomenberg *et al.,* 1994). Hemorrhagic cystitis may also develop following renal transplantation (Koga *et al.,* 1993). A relatively infrequent post-transplantation complication is adenovirus hepatitis mainly caused by serotypes 1, 2 and 5 (Cames *et al.,* 1992). Approximately 10–12% of AIDS patients excrete adenoviruses group B in urine, stool or develop necrotizing hepatitis of adenovirus etiology (DeJong *et al.,* 1983; Horwitz *et al.,* 1985; Krilov *et al.,* 1990; Janoff *et al.,* 1991). Adenoviruses D were predominantly isolated from cases of persistent diarrhea of HIV-positive homosexual patients. Several adenovirus serotypes were isolated from AIDS patients with intestinal dysfunctions including the new types (43–47) of the D species (Hierholzer *et al.,* 1988). In congenital agammaglobulinemia, adenovirus-associated necrotizing hepatitis and/or interstitial pneumonia were described (Siegel *et al.,* 1971). Similar complications may accompany a severe thymus hypoplasia (Wigger and Blanc, 1966). The increased susceptibility to acute primoinfection in patients who underwent ISP treatment is an interesting issue. Even though the probability of primoinfection is not enhanced in ISP patients, the course of disease is more severe. The acute primoinfection results either in pneumonia or in hepatitis (Zahradnik, 1980).

Finally, it should be mentioned that the E1 region of the genome of oncogenic adenovirus A species may become integrated into host cell chromosomes. However, the human cells are not transformed unlike to the rat, hamster, and mouse cells (Shenk, 2001). The exact mechanism, how E1A and E1B polypeptides of oncogenic adenoviruses promote transition from the G1 to S phase is not quite clear. Their interaction with cellular regulators p53 and Rb was firmly established. Even more complex is the explanation of the absence of oncogenic action of adenovirus oncoproteins in human cells. The different behavior of oncogenic adenoviruses in human cells is usually attributed to the lack of telomerase expression (Hahn *et al.,* 1999).

4.9. Hepatitis B virus (HBV)

In humans, viral infections of the liver are caused by four viruses namely Hepatitis A virus (family *Picornaviridae*), Hepatitis B virus (HBV, family *Hepadnaviridae*), Hepatitis C virus (HCV, family *Flaviviridae*) and Hepatitis E virus (family *Caliciviridae*). Two of them, HBV and HCV show the tendency to undergo latency causing various forms of chronic infection, which reactivate under ISP conditions. Since this survey concerns DNA viruses, our considerations will be restricted to HBV. During standard healing of acute hepatitis B, many Th lymphocytes recognize HBV-specific antigenic peptides. They produce an excess of Th 1 cytokines IL-2, IFN-γ, and TNF, which promote the proliferation of Tc lymphocytes. Before effective destruction of infected target cells, the main mechanism of blocking HBV replication is IFN-α/β production. Hepatocytes expressing HBs/L, HBc/C antigens and viral DNA polymerase interact with TCRs of T cells (Zarsky *et al.,* 2002). The hallmark of productive HBV replication in the liver is the appearance of HBs/L (complete Dane particles enveloped with the large surface glycoprotein) in blood along with the HBe antigen, vDNA, and viral DNA polymerase. A sign of recovery is the production of anti-HBe antibodies with elimination of HBe antigen and the presence of anti-HBsAg class IgG antibodies with elimination of HBsAg. The HBe antigen that is a polypeptide split off from the nuclear HBc/C protein is present in blood not only during the acute phase, but also during productive reactivation of chronic HBV infection, which may occur in 2–10% of HBV infected subjects. At least two different forms of chronic hepatitis B exist. One of them is the HBs/S antigenemia with incomplete HBs/S (Australian antigen) in blood and persisting HBc/C antigen in the liver. There is no HBe antigenemia and the level of HBV DNA in the plasma is low. In the succeeding form of chronic active hepatitis B the viral DNA in blood increases along with the appearance of HBe antigen (Hollinger and Liang, *et al.,* 2001). However, in certain forms of chronic active hepatititis B, there is no correlation between increased HBV DNA levels and the appearance of HBe. The HBs/S and HBs/L antigen carriers having high HBV DNA levels but no HBe do not develop anti-HBe antibodies. It is assumed that this form of chronic active hepatitis comes from a mutation of the pre-C polypeptide; the liver does not secrete HBe, since it cannot be split off from the mutated HBc/C protein (Roingeard *et al.,* 1990; Korenblat and Dienstag, 2002). For this reason, HBe antigenemia may not be the main criterion of virus reactivation in chronic HBsAg carriers.

The iatrogenic ISP increases the tendency to produce more HBc and complete infectious Dane particles in the chronic HBs carriers. Recipients, who had chronic HBs antigenemia before transplantation have to be monitored for possible increase of blood HBV DNA levels. Virtually any recipient with chronic active hepatitis may progress to liver cirrhosis within 5–10 years post-transplantation, but there is no clear-cut criterion predicting the outcome except of HBV DNA motoring (Davies *et al.,* 1995). Not only the course of chronic hepatitis B may be more severe following

Table 6. Transplantation strategy from the viewpoint of hepatitis B infection

A. The donor of grafted tissue

1. The liver donor should not be HBsAg-positive. The HBsAg-negative donor should not have antibodies against HBc/C antigen. The donor, however, may be positive for HBs antibodies, since anti-HBs antibody is the sign of vaccination.
2. If the HBsAg negative donor has anti-HBc antibodies (a hallmark of previous HBV infection), his bone marrow and/or kidney may be used for transplantation. The HBsAg negative and anti-HBs positive person is certainly not a chronic HBV carrier.

B. The recipient of grafted tissue

1. The HBsAg positive recipient should be tested negative for HBV DNA and HBe antigen. Such patient may undergo bone marrow transplantation provided that he is monitored for reactivation of chronic HBV infection. If DNA viremia (and/or HBe antigenemia) occurs, the post-transplantation ISP regimen should be withhold.
2. The possibility of the reactivation of chronic hepatitis B in the HBsAg positive but HBe negative recipient is higher following renal transplantation than following bone marrow transplantation.
3. Transplantation of liver from a healthy donor to HBsAg positive patient with chronic active hepatitis and/or liver fibrosis is regular provided that the HBV DNA level of the recipient will be monitored and his possible HBe antigenemia has been successfully treated. The drugs of choice are lamivudine and/or recombinant IFN-α treatments in combination with specific immunoglobulin. In addition, the recombinant HBs/S vaccine may be administered.
4. The bone marrow and/or kidney recipients should be monitored for HBV DNA and treated for HBe antigenemia.

Notice: General rules for standards of quality, safety of donation, testing, procurement, processing, preservation, storage and distribution of human tissues and cells were set by the Directive 2004/23/EC of the European Parliament and the Council on March 31, 2004. The EU rules set by the Council Directive 2006/86/ES were published in Slovak republic on October 25, 2006 and confirmed by the Act 20/2007 of the Slovak National Goverment on December 20, 2006.

renal transplantation, but also the probability of rejection episodes may be higher (Grekas *et al.,* 1995). The probability of acute rejection episodes increases approximately by twofold.

Kidney donors considered as relatively safe should be HBsAg negative, if recovered from hepatitis B and never showed HBs antigenemia even they are HBc antibody positive. It is recommended to avoid HBsAg positive kidney donors, especially when the recipient is HBs antibody negative. About 10% of the HBs antibody negative recipients may develop chronic HBsAg carriership. HBsAg antigenemia would transiently occur in about 25% of all transplant recipients, whose donors were HBsAg positive. Therefore, such recipients should be immunized with the HBs/S vaccine before transplantation. The HBs antibody positive and HBs/S immunized bone marrow recipients seem to be better protected, when receiving the bone marrow from an HBsAg positive donor (Lau *et al.,* 1999). Summing up, the HBs antibody negative transplant recipients should be vaccinated, when receiving bone marrow or kidney from an anti-HBc positive HBsAg carrier.

Even more precautions are necessary, when selecting the donors for liver transplantation (Table 6.). The use of a liver from the HBs antibody positive person is not forbiden. However, many authors prefer liver coming from a donor who never had viral hepatitis B or C (Rosen and Martin, 2000). Liver transplant recipients are rarely HBsAg negative, since chronic infection with HBV or HCV or both is a frequent reason for developing liver fibrosis or cirrhosis. Before the transplantation, HBsAg positive recipients should be treated with drugs inhibiting replication of HBV (Zaia, 2002). Briefly, lamivudine in combination with specific immunoglobulin and recombinant IFN-α is most frequently used (Perillo *et al.,* 2001). Famciclovir, adefovir or ganciclovir are the alternative inhibitors of the viral DNA polymerase in the case of resistance to lamivudine.

The chronic HCV infection in association with the transplantation should be only briefly mentioned, because hepatitis B can be combined with hepatitis C. In double infected patients, the disease has a more severe outcome. After bone marrow or renal transplantations, the probability of transition from chronic active hepatitis to chronic fibrosis is about 67% (Puteil-Nobel *et al.,* 1965). Chronic liver fibrosis associated with HBV and/or HCV may be the cause of liver failure at a frequency o 7% or 17%, respectively (Gane *et al.,* 1996). In general, within 5 years post-transplantation, liver cirrhosis would occur in all recipients, who had chronic hepatitis B and C before the onset of ISP regimen.

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