

**Conclusion:** We propose that tissue destruction plays a key role in the initiation of immunity and that the *prevention* of tissue destruction has been a selective pressure for the evolution of the adaptive immune system.

#### P.4.09.19 **Positive selection mediated by cytotoxic T lymphocytes influences HIV-1 evolution during primary infection**

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**Introduction:** A specific cytotoxic T lymphocyte (CTL) response is elicited early in many viral infections, and is frequently followed by successful elimination of the virus. In acute HIV-1 infection, the development of the CTL response coincides with the decline of the massive early viraemia that is characteristic of the first few weeks of this infection, but the virus is not eradicated altogether. In order to address the possibility that viral escape from CTL recognition may be a factor in the failure of the immune system to clear the virus, we aimed to characterize the nature of the persistent virus with respect to the emergent CTL response in donors with primary HIV-1 infection.

**Materials and Methods:** We performed a detailed genetic analysis of proviral diversification in serial blood samples from a donor with primary HIV-1 infection who generated a strong CTL response to a HLA B8-restricted peptide epitope (FLKEKGGL) in Nef.

**Results:** Clear evidence of positive selection of proviral sequences encoding variants within this epitope was obtained. These variants either diminished, or escaped, recognition by the patient's anti-Nef CTL. Further evidence of positive selection in this donor came from the observation of epitope deletions following the onset of the CTL response.

**Conclusion:** This study provides evidence that escape from CTL recognition may be a crucial event which allows viral persistence in HIV-1 infection.

#### P.4.09.20 **Inhibition of ubiquitin-proteasome dependent protein degradation by the Gly-Ala repeat domain of the Epstein-Barr virus (EBV) nuclear antigen (EBNA)-1**

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**Introduction:** The Epstein-Barr virus (EBV) encoded nuclear antigen (EBNA)1 is expressed in latently EBV infected B-lymphocytes that persist for life in healthy virus carriers and is the only viral protein regularly detected in all EBV associated malignancies. We have shown that the internal Gly-Ala repeat region of EBNA1 generates a cisacting inhibitory signal that interferes with MHC class I restricted presentation (Levitskaya et al., 1995, *Nature*). This constitutes a previously unknown mechanism of viral escape from CTL surveillance, and supports the view that the resistance of EBNA1 expressing cells to CTL mediated rejection is a critical requirement for EBV persistence and pathogenesis. MHC class I restricted presentation of endogenous antigens required degradation by the ubiquitin/proteasome pathway. In order to test whether the Gly-Ala repeats may interfere with this process we have compared the degradation of EBNA1, EBNA4 and EBNA4 chimeras containing Gly-Ala repeat sequences in *in vitro* degradation assays.

**Material and Methods:** *In vitro* translated proteins were subjected to degradation in a rabbit reticulocyte lysate dependent system.

**Results:** EBNA4 was degraded in an ATP/ubiquitin/proteasome dependent manner. EBNA1 failed to undergo proteolytic degradation whereas an EBNA1 deletion mutant devoid of the Gly-Ala repeats was efficiently degraded. Conversely, insertion of Gly-Ala repeat sequences of different length or in different positions of EBNA4 molecule led to inhibition of protein degradation. A weaker inhibitory effect was achieved by insertion of a Pro-Pro-Ala repeat.

**Conclusion:** The results suggest that the internal repeat domain of EBNA 1 prevents antigen presentation by inhibiting proteasome dependent processing. The repetitive nature of the domain rather than its aminoacid content may be important for the effect.

#### P.4.09.21 **Identification of the B-cell superantigen binding site of HIV-1 gp120**

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The gp120 envelope protein of HIV-1 is able to bind membrane IgM on normal human B cells and to induce their activation in a VH3 immunoglobulin (Ig) gene-family-specific manner. Since this VH gene family is the largest in the hu-

man repertoire, this superantigen (SAG) property is thought to have deleterious consequences for the host, including a progressive decline of B cells with progression of the disease. We have identified the epitopes on the gp120 molecule engaged in B-cell SAG interactions.

The sequence motifs on gp120 involved in SAG binding to normal Igs were mapped by competitive immunoassays, using synthetic peptides covering the whole sequence of gp120.

We show that the SAG binding activity is present in gp120s from highly divergent isolates of HIV-1 belonging to clades derived from various geographical origins and the SAG binding site is formed by protein sequences from two region of the gp120 molecules. The core motif is a discontinuous epitope spanning the V4 variable domain and the amino-terminal region flanking the C4 constant domain. The most critical residues appear to be Leu<sup>395</sup>-Asp<sup>397</sup> and Ile<sup>425</sup>-Gln<sup>427</sup>. Residues from the C2 constant domain (positions 252-272) seem also to play an accessory role in SAG binding of gp120 to normal human Igs.

The identification of the superantigen binding site of gp120 is potentially important in the design of a successful gp120-based vaccine against HIV-1.

#### P.4.09.22 **"Murine" toxin as superantigen: The computer forecast and experimental confirmation**

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**Introduction:** Despite prescription of opening and duration of learning of the *Yersinia pestis* "murine" toxin (MT), the mechanism of its influence on an organism of the sensitive owner remains practically not clarified. In the given message the results of the computer analysis of an aminoacid sequence of the *Y. pestis* MT-protein, which have allowed to assume an availability at it of a superantigenic activity (SA), and also experimental confirmation of the computer forecast are represented.

**Materials and Methods:** MT has been isolated from *E. coli* DH5 carrying pMB188 recombinant plasmid with the cloned MT-gene. The homogeneity of the preparation was evaluated in HPLC system (Gilson) and by SDS-PAAG electrophoresis. The nucleotide sequence of a gene and the aminoacid structure of MT-protein were investigated with the use of the author's algorithms of applications DHAis (Hitachi) and Gene Pro (Hoefel Sci.Instr.). SA activity of the MT preparation was evaluated by its capacity to cause polyclonal activation of T-lymphocytes, to induce cytokines (IL-1, IL-2, TNF-alpha) production and also by the dependence of its recognition by T-lymphocytes on Ia-antigen (Ia-a) on accessory cells.

**Results:** The computer analysis of an aminoacid sequence of MT-protein has revealed 74 amphipathic aminoacid residues. Their ability to form a stable structures in which the hydrophobic and hydrophilic residues tend to occur on opposite faces, is a critical requirement for a T-cell antigenic sites. Predicted epitopes were localized between residues 80-120, 235-250, 292-303 and 494-526. In structure first and second of them sequence motif X (D) (Q) NXXXXX (I) (S) X, characteristic for sequences, recognizing the sites of binding of a class II Main Histocompatibility Complex antigens is traced. Revealed antigenic sites on the MT-polypeptide allow to assume an availability at it of the properties, characteristic for superantigens. In experimental researches is established, that MT induces T-lymphocyte proliferation more efficiently than ConA and PHA used at a concentrations 100-200-fold higher than that of MT. MT caused pronounced shift in the proportion of T-lymphocyte subpopulations with decline of T-helper and an increase of T-suppressor cells. Cytokine-inducing activity of MT has also been elicited. In contrast to mitogen, the ability of MT to induce polyclonal activation of T-lymphocytes could be blocked by monoclonal antibodies to Ia-a.

**Conclusion:** The experimental data have confirmed the computer forecast about an availability of the SA at MT. It is possible to assume, that the powerful activity of MT to stimulate of T-cells, leading to a release of lymphokines, which stipulate in high concentration the immunopathological reactions, determines its toxicity and a role in pathogenesis of a plague.

#### P.4.09.23 **Inability of antimitogenic IgG to prevent erythrocytic toxin-induced arteritis in the rabbit ear experimental model**

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**Introduction:** Erythrocytic toxins (ET) are well-known superantigens (SAG) of streptococcal origin that induce a wide range T-cell activation via MHC and TCR V  $\beta$  binding. On the other hand ETs can induce a subacute type arteritis by local intracutaneous injection in our rabbit ear experimental model. Stimulation with SAGs may result in aberrant and dangerous "immune" reaction *in vivo*. How can it be possible to prevent the host from SAGs?

**Materials & Methods:** The rabbit ear experimental model developed by us was employed. A quantitative evaluation of histological findings of arterial lesions was made.