Short Communication

Identification of Pterin Binding Sites on Class I HLA Molecules Associated with the Interleukin 2 Receptor

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Introduction

It is well established that the proliferation of T lymphocytes is initiated by binding of interleukin 2 to specific high affinity sites of the membrane. These receptors are composed of at least two polypeptide chains, p55 and p75 (cf 1). Lymphocyte derived H₄biopterin is a feed-back modulator which affects the formation of the high affinity receptor complex. This results in an increased affinity to the ligand (2) and modulates various aspects of IL-2 signal transmission. This finally results in an enhanced entry of the T cells into the DNA synthesizing phase (cf 3).

There is accumulating evidence that in addition to p55 and p75, class I HLA molecules associate with the receptor complex (cf 1). This association was confirmed in our experiments by affinity labeling of the IL-2 binding polypeptides in the HTLV-I transformed T helper cell line MT-2. Their separation by SDS-PAGE revealed a chain with an apparent molecular weight of about 60 kDa. It could be precipitated by the monoclonal anti-HLA-A,B,C antibody W6/32 (data will be presented elsewhere).

This report provides evidence that class I HLA molecules have pterin binding sites so that they mediate the modulatory effect of H₄biopterin. The anti-idiotypic monoclonal antibody NS7 is known to react with pterin binding sites on a number of enzymes which use pterins as cofactors or substrates (4). It was used as a tool for probing pterin binding sites on class I HLA molecules of MT-2 cells. In many cases anti-idiotypic antibodies may mimic the action of a receptor binding component. We therefore examined a H₄biopterin mimicking effect on receptor affinity to its ligand IL-2.

Results

Two antibody sandwich assay

Plastic wells were coated with the monoclonal polymorphic antibody W6/32 which reacts with class I HLA-A,B,C. By this means, class I HLA molecules in Nonidet P-40 lysates of MT-2 cells were selectively captured and fixed to the wells. Their potential pterin binding sites were probed with the anti-idiotypic antibody NS7. This IgM type antibody was finally quantified by peroxidase-linked anti-mouse IgM. Figure 1 demonstrates that the pterin binding sites increase in correlation with class I HLA antigen.

Figure 1. Two antibody sandwich assay with lysates of MT-2 cells. Class I HLA molecules from cell lysate were allowed to react with the anti-HLA monoclonal antibody W6/32 which had been fixed to microtiter plates. The pterin binding sites of the class I HLA antigen were then determined by the anti-idiotypic monoclonal antibody NS7. Daudi cell lysates (which lack class I HLA antigen) served as a control for unspecific reaction. Dilution of the W6/32 antibody: ●—● 1 : 200; △---△ 1 : 400; x---x 1 : 600.

Pterin conjugate/antibody sandwich assay

H₄biopterin was conjugated to bovine serum albumin (5) and the conjugate was coated to plastic wells. By this means, pterin binding polypeptides in Nonidet P-40 lysates of MT-2 cells were captured and fixed to the wells. They were probed with the anti-class I HLA antibody W6/32 which was then quantified by peroxidase-linked anti-mouse IgG. Figure 2 demonstrates that polypeptides which had bound to the pterin are identified as class I molecules. Also membrane lysates of MT-2 yield a positive reaction. Moreover, MT-2 cells lack aromatic amino acid hydroxylases (unpublished results).

Figure 2. Pterin conjugate/antibody sandwich assay with lysates of MT-2 cells. H₄biopterin binding polypeptides from the cell lysate were allowed to react with a H₄biopterin-BSA conjugate which had been fixed to microtiter plates. Class I HLA antigen was then determined by the monoclonal antibody W6/32. BSA coating served as a control for unspecific binding. Unspecific staining was determined by Daudi cell lysates and was subtracted.

Together with the results obtained by the two antibody sandwich assay, the data suggest that membrane class I HLA antigen is the major pterin binding polypeptide in MT-2 cells.

Modulation of IL-2 receptor affinity by the pterin mimicking antibody NS7.

Equilibrium binding of ¹²⁵I-IL-2 to MT-2 cells and Scatchard plot analysis of the data by the LIGAND program was performed as earlier described for the

murine cytotoxic T cell clone CTLL-2 (2). The data indicated a K_d value of 7.12×10^{-11} M IL-2 with a 95% confidence interval from $5.98 - 8.85 \times 10^{-11}$ M IL-2. Similarly to CTLL-2 cells, inclusion of H₄biopterin (6×10^{-7} M) during equilibrium binding increased the apparent affinity of the receptor and reduced the K_d to about one half. Addition of NS7 antibody during the binding period also reduced the K_d value of the MT-2 cells from 7.12×10^{-11} M to 4.44×10^{-11} M IL-2 with a 95% confidence interval from $3.71 - 5.32 \times 10^{-11}$ M IL-2.

This data show that the antibody NS7 which is an internal image anti-idiotypic antibody for reduced pterin molecules, not only mimics them in pterin-dependent enzymes (4) but also mimics as an IL-2 receptor binding component the effect of H₄biopterin and modulates the formation of the receptor complex.

The association of pterin binding class I HLA molecules with the IL-2 receptor (see Introduction) makes them the most likely candidate to mediate the effect of H₄biopterin on receptor affinity. The IL-2 segment which binds to class I HLA antigen and the modulation of this interaction by the pterin are under investigation.

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