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AUTOANTIBODIES FROM ITP PATIENTS RECOGNIZE HYDROPATHICALLY GENERATED EPITOPES

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The antigenic determinants involved in autoantibodies binding to platelets in chronic Immune Thrombocytopenic Purpura (ITP) are still unknown. Recent works implicate, however, integrin GP IIb/IIIa as the target antigen in antibody mediated platelet destruction (Bussel, 1990, *Hematol. Oncol. Clin. N. Amer.*, *4*: 179-191). Accurate identification of epitopes involved in such interactions would have clinical relevance.

Our laboratory has recently used the hydropathic complementarity principle to characterize the fibronectin binding domain in the GP IIIa molecule (R. Pasqualini et al., 1989, *J. Biol. Chem.*, *264*: 14566). This principle states that peptides resulting from the transcription of complementary DNA strands would bind one another. Experimental support for this concept has emerged from many laboratories and the chemical plausibility as well as the evolutionary implications of such interactions have already been discussed (R. R. Brentani, 1988, *J. Theor. Biol.*, *135*: 459; *J. Mol. Evol.*, *3*: 239). We showed, that peptides predicted from the complementary nucleotide sequence to the one that codes for the RGD domain in rat and human fibronectin (WTVPTA and GAVSTA, respectively) can block the ligand/receptor interaction (R. Pasqualini, *loc. cit.*). Additionaly, a serum made upon immunization with WTVPTA was capable of binding to GP IIIa in Western blots.

Since autoantibodies seem to be directed towards important protein domains, it seemed attractive to test whether GPIIb/IIIa recognizing sera from ITP patients, could bind to these hydropatically deduced peptides.

Sera from 30 patients with clinically defined ITP were tested in ELISA for reactivity against WTVPTA and affinity purified GPIIb/IIIa. Seventeen sera reacted strongly with the integrin, 5 of which produced high ELISA optical densities when tested for reactivity towards the peptide. Although a moderate reactivity against both antigens was seen in almost all tested sera, antibodies from 3 patients reacted strongly with GP IIb/IIIa but did not react with the peptide.

In order to establish a correlation between anti-peptide and anti-GP IIb/IIIa activity, the 27 sera that had produced positive ODs against WTVPTA were submitted to a linear regression analysis by the least squares method. It showed a direct correlation ($r = 0,71$) between activity against the two antigens. The same analysis performed using the ODs produced against an irrelevant aminoacid sequence did not show a significant result ($r = 0.05$). This finding suggests a close relationship between WTVPTA and the complex GP IIb/IIIa indicating that the hexapeptide can mimic an epitope within the integrin molecule.

We next used serum from ITP patient to further analyze this correlation. This serum produced positive ELISA curves against GP IIb/IIIa and against the hexapeptide WTVPTA. WTVPTA-specific autoantibodies, purified from this serum using a WTVPTA-Sepharose column, reacted with GP IIb/IIIa in a direct binding assay. The same pool of autoanbibodies was also able to recognize the GP IIb/IIIa corresponding band in Western blots of platelet membrane extracts. These assays confirm that WTVPTA recognizing autoantibodies are within the population that binds to the GP IIb/IIIa molecule.

Binding of the peptide specific autoantibodies to WTVPTA was dependent on an appropriate hydropathic profile of the hexapeptide, as seen by liquid phase competition experiments. The interaction cited above was inhibited by GAVSTA (hydropathically similar to WTVPTA) but not by GAGSTA, which display a different hydropathic profile.

In summary, our data confirm our previous demonstration that the hexapeptide WTVPTA can mimic an important domain in the GP IIb/IIIa molecule. We believe that this domain can represent the fibronectin binding site but additional experiments are in progress in order to confirm this suggestion.