Neutralization of the Biological Activity of Glycosylated and Non-Glycosylated hGM-CSF by Monoclonal Antibodies

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1. Introduction

Human Granulocyte Macrophage-Colony Stimulating Factor (hGM-CSF) is a glycoprotein which has clinical utility by enhancing the rate of hematopoietic recovery after cancer chemotherapy. Nevertheless, there is increasing evidence of spontaneous human anti-GM-CSF antibodies associated with chronic infections, autoimmune diseases or simply their occurrence in normal sera that could compromise the clinical efficacy of the therapy. The specificity of these antibodies should play an important role in the failure or success of GM-CSF clinical trials.

In this work we show a bioassay based on the inhibition of the biological activity of glycosylated and non-glycosylated GM-CSF using three monoclonal antibodies (MAbs M1B8, CC5B5 and M7E10). The binding sites of the MAbs were mapped using sets of overlapping peptides as well as generic hexapeptide libraries prepared by SPOT synthesis technique.

2. Materials and Methods

2.1. MAbs Production, Purification and Affinity Characterization

The MAb producing murine hybridoma clones were established following immunization with pure E. coli-derived GM-CSF with the use of standard fusion protocols (Galfré and Milstein). Hybridomas were grown in mice to produce ascitic fluid and MAbs were purified by protein A affinity chromatography. The affinity constants of the anti-GM-CSF MAbs were determined as described by Friguet et al.

2.2. Mapping of GM-CSF-Derived Epitopes by SPOT Synthesis

All cellulose-bound sets of peptides (peptide scans and libraries) were automatically prepared according to standard SPOT synthesis protocols (Frank). Combinatorial hexapeptide libraries were prepared to delineate peptide sequences that were recognized by the antibodies and mimicked conformational epitopes (mimotopes). The following strategies were used: a dual positional scanning with 2,000 hexapeptides in one single screen and a second and third generation of an iterative search, starting with two defined positions.
Figure 1. Space filling representation of the 3D-structure of native hGM-CSF.

(400 hexapeptide library). An overlapping peptide scan (peptide length 15 aa, offset of 3 aa) spanning the GM-CSF primary sequence followed by electroblotting (Rüdiger et al.) was also performed. The analysis of peptide bound antibodies were carried out as described by Frank and Overwin using a chemiluminescence detection system.

2.3. INHIBITION OF THE BIOLOGICAL ACTIVITY BY MAbs

A bioassay based on the TF-1 cell line, which proliferates in response to GM-CSF, was used. Purified MAbs were diluted serially with RPMI 1640 containing 5% fetal calf serum (FCS) and preincubated for 1 h at 37°C with equal volume of E. coli-derived GM-CSF (6.25 U/ml) or CHO-derived GM-CSF (6.25 U/ml). Exponentially growing TF-1 cells resuspended in the same culture media were added to each well (10,000 cells/well). The plates were incubated for 48 h and cell proliferation was determined by measuring the dehydrogenase enzyme’s activity with a commercial colorimetric kit (Cell Titer 96™, Promega, USA).

3. Results and Discussion

Dual-positional scanning of a combinatorial hexapeptide library and subsequent iterative searches with two defined positions showed that MAb CCSBS bound peptides AERRF and RERW, probably mimicking the conformational dependent epitopes A18E21R23R24F119 and R23E21N17W13.

MAb MIB8 bound peptides PFEWE, FFEWE and WFEWE. These peptides might mimic the non-linear sequence P118F119W13E14 in the GM-CSF molecule. Taking into account the three-dimensional structure of the native GM-CSF molecule, we observed that the non-linear epitopes bound by both MAbs were very closely located on the protein surface (Figure 1). Finally, the overlapping peptide scan followed by electroblotting of bound antibodies described a discontinuous epitope recognized by MAb M7E10, where the peptide L61YKQGLRSLTK72 could be part of this assembled epitope (Figure 1). Table 1 summarises the GM-CSF results of each MAb. MAbs M1B8 and CC5B5 showed higher neutralizing activity of the non-glycosylated GM-CSF-induced proliferation. This behaviour was in agreement with the lower affinity of both MAbs for the glycosylated