To the editor:

Differences between the N-glycans of human serum erythropoietin and recombinant human erythropoietin

The differences between the N-glycans of human erythropoietin (hEPO), extracted from serum, and recombinant human erythropoietin (rEPO) reported by Skibeli et al\(^1\) have received attention in the editorials of 2 other journals. One suggested that these differences might be involved in the etiology of the autoimmune pure red cell aplasia found in some patients treated with rEPO.\(^2\) The other suggested that these differences might form the basis for a test to detect the doping of athletes with rEPO.\(^3\) A review of the data of Skibeli et al, however, indicates that the differences they have reported between the N-glycans of hEPO and rEPO are not consistent with the differences between the physicochemical properties of these EPOs found in other laboratories. This discrepancy may be a consequence of the effects on the N-glycans of hEPO of the conditions used by Skibeli et al to extract hEPO from serum in order to compare it with unextracted rEPO.

Skibeli et al reported that hEPO lacked the tetra-acidic (tetrasialylated) N-glycans found in the rEPOs, and was also lower in its content of tri-acidic N-glycans.\(^1\) These findings are summarized in their Table1,\(^1\) which also permits comparison of the N-glycan charges of different EPOs, as calculated, for example, by the method of Hermentin et al.\(^4\) This suggests that the total negative charge of the N-glycans of hEPO is \(\sim 70\%\) that of the rEPOs, and implies that hEPO is less acidic than the rEPOs. Thus the sialic acid residues of the N-glycans of EPO represent up to 12 of a possible total of 14 sialic acid residues in EPO, and make a major contribution to the net negative charge of EPO, as indicated by the fact that the pI of intact EPO is in the range of about 2.5 to 4.0,\(^5\) and the pI of desialylated EPO is about 8.5.\(^6\) However, other studies have consistently shown hEPO to be more acidic than rEPO,\(^7,\) and, indeed, this is the basis for an established test for doping.\(^9\)

The discrepancy between the findings of Skibeli et al and those of others is probably due to the conditions used to extract the hEPO from serum to compare it with unextracted rEPOs. Although the data in Figure 8 of Skibeli et al is said to demonstrate “the similarity of sugar profiles from rEPO with or without a bead-extraction step,” comparisons of the areas under the 2 elution profiles indicates that the extraction procedure has reduced the recovery of all oligosaccharides with elution times of 60 minutes or more, representing tri- and tetrasialylated N-glycans, and has increased the relative recovery of most of the neutral, mono- and di-sialylated N-glycans.\(^7,\) This effect of the extraction procedure probably represents some desialylation of the N-glycans of EPO, since the 20min-treatment with 10mmol/l HCl, used to dissociate the antibody-bound EPO, is also commonly used, albeit at 80°C rather than ambient temperature, for the quantitative desialylation of N-glycans.\(^10\)

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References


Response:

Inherent charge properties of the isolated sugar parts of human serum erythropoietin

Drs Storring and Yuen claim our data\(^1\) to be inconsistent with other reports describing the molecular differences between endogenous human erythropoietin (hEPO) and recombinant human EPO (rEPO) due to desialylation of human serum EPO caused by the extraction conditions used in our study. But they do not take into consideration that we analyzed EPO from human serum, while the reports they refer to\(^2-6\) all investigated EPO from human urine, a completely different matrix.

This is important as charge profiles of glycoproteins undergo changes during renal secretion.\(^7,\) In fact, 2 of the papers Storring and Yuen refer to\(^7,\) described human serum EPO as more basic than human urinary EPO when analyzing the intact glycoprotein by isoelectric focusing. Furthermore, one of the other papers referred to as contradictory to our study\(^7\) showed a more basic charge pattern of human urinary EPO than the one obtained for rEPO. In addition, Storring has used this urinary EPO preparation in his own work when comparing the isoelectric pattern of different batches of rEPO with human urinary EPOs,\(^4\) reproducing the findings of Imai et al.\(^3\) Storring and Yuen also mentioned the shift in isoelectric point to 8.5 caused by the desialylation of hEPO,\(^3\) as an illustration of the contribution of sialic acids to the net charge of hEPO without
commenting that Imai et al used recombinant hEPO and not endogenous hEPO.

Furthermore, during discussion of our results, Storring and Yuen did not take into consideration the fact that our findings of a reduced sialylation of the glycans of human serum EPO referred to the isolated sugar part, which cannot be directly compared to studies of the charge pattern of the intact glycoprotein. This point has previously been elaborated by Tsuda et al, who reported that the glycans from rhEPO contained more sialic acids than glycans from human urinary EPO, indicating that sugar from human urinary EPO is more basic than sugar from rhEPO.

In addition, we have presented results obtained from the analyses of serum EPO from anemic patients that must be taken into consideration when interpreting our results. In our paper all relevant reports, including the papers mentioned by Drs Storring and Yuen, were thoroughly referred to and discussed.

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To the editor:

Should patients with FMF undergo BMT?

Recently, Milledge et al reported a patient suffering from both CDA (congenital dyserythropoietic anemia) and presumptive FMF (familial Mediterranean fever). They observed that the patient’s symptoms of “FMF” rapidly abated after BMT (bone marrow transplantation) that was required to treat her CDA. This prompted the authors to conclude that through BMT the missing factor in FMF was provided.

In addition, we have presented results obtained from the analyses of serum EPO from anemic patients that must be taken into consideration when interpreting our results. In our paper all relevant reports, including the papers mentioned by Drs Storring and Yuen, were thoroughly referred to and discussed.

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Response:

Bone marrow transplantation for FMF

Dr Touitou raises questions about the diagnosis of FMF in our patient and the temporal relationship between disappearance of symptoms and the transplantation.

The diagnosis of familial Mediterranean fever (FMF) in our patient was initially a clinical one, although perhaps her disease might better be described as familial paroxysmal polyserositis rather than FMF, as it was the characteristic recurrent serositis rather than the fever that led to the clinical diagnosis at 14 months of age. Her clinical presentation was given in brief in the case report, but we are happy to expand on this here. She presented with recurrent joint swellings of the elbows and knees, which were asymmetrical. She showed exquisite sensitivity to colchicine, with

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References


Moreover, from the genetic and clinical data described in this report, this little girl might suffer from an hereditary inflammatory disorder other than FMF, for example hyper-IgD syndrome (HIDS) or chronic infantile neurologic cutaneous and articular syndrome (CINCA), and be simply a carrier for Met680Ile, the most frequent FMF mutation in the Egyptian population. Finally, I would like to mention that the MEFV-encoded protein contains 781 amino acids (not 791, as written in the introduction) and that the “Asp692Ile” mutation does not exist and was probably meant as the rare “Ile692del” deletion (mutation analysis).

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References