To the Editor:

The gene for thrombopoietin (Tpo) has been cloned and the availability of recombinant material for laboratory experiments and patient care is imminent. Initial experiments from several laboratories have suggested that different forms of Tpo are produced that vary in both polypeptide and carbohydrate composition and have different activities in both in vitro and in vivo settings. Therefore, it is important to establish a standardized method for calculating units of the hormone so that products and experiments from different laboratories can be compared. We propose that Tpo units be based upon an in vivo measurement of activity, and be similar in magnitude to the units that are now being used for erythropoietin (Epo).

Early in the history of Epo characterization, workers used an
exhypoxic polycythemic mouse assay for the measurement of laboratory standards. This assay relied upon measuring $^{35}$S incorporation into red blood cells of mice after removal from hypoxic environments and injections of test substances. Several reference laboratories participated in establishing an international Epo standard, and this material was useful in comparing Epo products from several different laboratories and commercial producers. Therefore, our suggestion is that workers in the Tpo field use similar methodologies for establishing a standardized international unit for Tpo.

The immunothrombocythemic mouse assay for Tpo, described in 1973, is based upon physiologic mechanisms similar to the exhypoxic mouse assay for Epo. This assay has been used successfully for establishing Tpo units in plasma and serum from thrombocytopenic animals and patients, and in partially-purified preparations of Tpo from human embryonic kidney cells. The assay measures $^{35}$S incorporation into platelets of mice that are administered a test substance during the rebound thrombocytopoietic phase of their response to acute thrombocytopenia. The advantage of this assay is that endogenous levels of Tpo are lower than normal during rebound-thrombocytosis, resulting in increased sensitivity to exogenous Tpo. A unit of Tpo is defined as the amount of material required to increase the percent $^{35}$S incorporation into platelets of immunothrombocythemic mice by 50% above baseline. A typical assay using partially-purified Tpo is shown in Fig. 1. The response in the assay is linear over a broad dose range, and units are easily assigned to test preparations.

This assay is an in vivo procedure, and results in the expression of units that are similar to the units presently assigned to Epo. Moreover, this method is specific for Tpo; it can determine Tpo levels in a variety of different body fluids (sera, plasma, and urine, along with extracts from liver tumors) and culture media, and provides a measure of activity in the most physiologically relevant setting. Although Tpo from several different species (human, rabbit, dog, bovine, goat, mouse, rat, and sheep) is active in the mouse, careful consideration will still need to be made in comparing results of different Tpo preparations.

Our suggestion is that units of Tpo be determined by the immunothrombocythemic mouse assay; this procedure can be used to accurately predict the amount of Tpo activity and, therefore, can lead to an international standard. After an international standard is established, in vitro studies using reference preparations can be performed, and comparisons of results can be made between laboratories on a standardized basis that is reflective of biologic activity.

REFERENCES


Defining units for thrombopoietin [letter]

TP McDonald, PS Sullivan and K Kaushansky

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