A randomized trial to evaluate the immunorestorative properties of thymostimulin in patients with Hodgkin's disease in complete remission

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Summary. A total of 19 Hodgkin's disease (HD) patients (12 male, 7 female) aged 26–67 years, who had been in complete unmaintained remission for 6 months or more when the study was initiated, were randomly given 50 mg thymostimulin (TS) i.m. daily (G1) or every other day (G2) for 35 days. A third group (G3) was not treated. Then TS, at the same dose was administered twice a week for the following 22 weeks in patients both initially receiving loading or intermittent TS treatment. When compared with age- and sex-matched controls, as a group, the patients' circulating levels of neopterin, type I and II IFN, beta-2 microglobulin (B2-M) and immunoglobulins (Ig) were also explored.

Introduction

The role of thymic polypeptides in the development of host T cell immunity is now widely accepted [28]. This view is substantiated by the in vitro and in vivo effects of thymic extracts on processes that lead to the phenotypic and functional maturation of precursor cells into immunocompetent cells [28]. In addition, thymic extracts have proved effective in restoring defective cell-mediated immunity, as well as in improving clinical factors in patients with primary and secondary immunodeficiencies [1, 4, 21, 22, 32, 35]. We have recently shown that thymostimulin (TS), a soluble extract of calf thymus, originally isolated and sex-matched controls, as a group, the patients' circulating levels of neopterin (a marker of macrophage activation (IL-2), gamma-interferon (IFN-y) and on in vivo serum IFN synthesis, to modulate the expression of beta-2 microglobulin (B2-M), a protein associated with class I histocompatibility antigens), [24] and to affect in vivo immunity, as well as in improving clinical factors in patients with primary and secondary immunodeficiencies [1, 4, 21, 22, 32, 35]. We have recently shown that thymostimulin (TS), a soluble extract of calf thymus, originally isolated and sex-matched controls, as a group, the patients' circulating levels of neopterin (a marker of macrophage activation whose synthesis is under the control of T cell lymphokines) [14]. The capacity of TS to induce in vivo type I and II IFN synthesis, to modulate the expression of beta-2 microglobulin (B2-M), (a protein associated with class I histocompatibility antigens), [24] and to affect in vivo immunoglobulin (lg) production were also explored.

In addition, since there is evidence that the schedule and duration of administration of thymic extracts may be relevant because of their immunomodulatory activities [17, 31], two different TS schedules were compared and a 22-week maintenance therapy evaluated.
Materials and methods

Patients. Immunological studies were performed in 19 HD patients (12 male, 7 female) between 25 and 67 years of age in complete unmaintained remission, and in a group of 48 reasonably well sex- and age-matched controls. At the time of diagnosis, the Ann Arbor staging was: 2 patients IA; 6 II A; 4 II B, 2 III A; 4 III B; 1 IV A. According to the Rye histological classification, 3 had lymphocytic predominance, 9 nodular sclerosis, and 7 mixed cellularity HD. Six patients had been treated with extended-field radiotherapy, 7 with polychemotherapy, and 6 with radiotherapy followed by chemotherapy. At least 6 months had elapsed between completion of therapy and the study initiation. The interval was 3 or more years in 7 patients, 2–3 years in 2, 1–2 years in 3, and less than 1 year in 7. All patients included in the study had depressed proportions (< 1 SD or <2 SD below the mean in controls) in three or all of OKT1 +, OKT3+, OKT4+, and E-AET+ cell subsets at two subsequent tests. This was the only immunological criteria for inclusion in the study.

Preparation of TS, (TP-1 Serona, Italy). Thymostimulin is a soluble calf thymus extract originally isolated by Falchetti et al. [5]. The methods for extraction and partial purification have been described elsewhere [2]. Briefly, minced calf thymus was treated with ammonium acetate. The extract obtained was precipitated with ammonium sulfate fraction obtained was precipitated with ammonium sulfate and gel-filtered on Sephadex G-50. This filtrate was then desalted on an Amion PM-10 membrane. This filtrate was then desalted on Sephadex G-25 and gel-filtered on Sephadex G-50. The final product, a group of polypeptides with a molecular weight of 1,000–12,000 daltons was lyophilized. The active fractions showed two characteristic bands with RF values of 0.22 and 0.42 at electrophoresis on polyacrylamide gel at pH 8.6.

TS administration schedules and toxicity. Patients were randomly assigned to one of two treatment groups or to a no therapy group. The treatment groups were as follows: Group-I (G1) received 50 mg TS i.m. at a daily loading dose for 35 days; Group-II (G2) received 50 mg TS i.m. every other day for 35 days; Group-III (G3) was not treated. TS was then administered in G1 and G2 twice a week for 35 days; Group-III (G3) was not treated. TS was then administered in G1 and G2 twice a week for 35 days; Group-III (G3) was not treated. TS was then administered in G1 and G2 twice a week for 35 days; Group-III (G3) was not treated. TS was then administered in G1 and G2 twice a week for 35 days. Group-I (G1) received 50 mg TS i.m. at a daily loading dose for 35 days; Group-II (G2) received 50 mg TS i.m. every other day for 35 days; Group-III (G3) was not treated. TS was then administered in G1 and G2 twice a week for 35 days. Full blood count, platelet count, liver enzymes (serum glutamic-oxalacetic and glutamyl transferase), and renal function tests were carried out before therapy, on day 35 and every 45 days thereafter. TS was fully tolerated and there was no hematological, hepatic or renal toxicity. Furthermore, no patient became pyrexial during TS treatment. In one patient, TS was discontinued after 35 days of administration because of cutaneous allergic reactions.

Preparation of peripheral mononuclear blood cells. Heparinized venous blood (mixed 3 to 1 with phosphate-buffered saline, PBS) was separated by Ficoll-Hipaque density centrifugation. Peripheral mononuclear blood cells (PMBC), recovered at the ring interface, were washed three times with PBS and then resuspended in RPMI 1640 medium (Grand Island Biological Co., Paisley, Scotland, UK) containing 24 mM hepes buffer, 2 mM L-glutamine, and gentamicin (50 μg/ml). This medium will be referred to as complete medium.