Thymopoietin and levamisole in chronic granulomatous inflammation

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SUMMARY Two animal models were used to investigate the mode of action of levamisole and thymopoietin. Neither compound influenced the subcutaneous carrageenan air pouch. While thymopoietin inhibited the formation of cotton pellet granulomas, levamisole enhanced it. This supports the conclusions of clinical studies reported elsewhere that levamisole and thymopoietin do not act in the same way in rheumatoid arthritis.

Levamisole was deliberately synthesised as a potent antihelminthic agent¹ and has been used both in human and veterinary practice. During veterinary use it was noted that host defence mechanisms were enhanced during treatment and that resistance to *Brucella abortus* was increased. Further experimental work confirmed enhancement of host responses by the drug.² These effects were similar in some respects to those of a thymic hormone—thymopoietin—whose isolation coincided with this work. The effect on E rosette forming cells both in mice and man was noted to be similar in response to both compounds and is discussed more fully with reference to thymopoietin.

Thymopoietin was first isolated from bovine thymus in 1974³ and was subsequently shown to be a 49 amino acid polypeptide chain.⁴ It has since been shown to be a selective inducer of thymocytes, enhancing T cell differentiation while inhibiting that of B cells.⁵ Effects on regulation of T cell function have also been demonstrated,⁶ and immune deficiency associated with neonatal thymectomy may also be partially corrected by the use of thymic hormone.⁷

Thymopoietin is also capable of restoring peripheral T cell counts to normal (recognised by the E rosette test) when depressed in the elderly and reversing the immune deficiency associated with thymic involution of old age. A single intravenous dose of thymopoietin is effective for 5 days.⁸ Thymic extract is known to increase the percentage of T cells

in immunodeficient children⁹ and thymopoietin itself to increase the percentage of T cells during influenza.¹⁰

In rheumatoid arthritis some patients have been noted to have low circulating levels of T cells, a deficiency that appears to be correlated with more severe forms of the disease. Some workers have shown to enhance concurrently the delayed hyper-levamisole.¹¹ Levamisole is undoubtedly beneficial in the management of rheumatoid disease and has been shown to enhance concurrently the delayed hypersensitivity response, thus being considered immunostimulatory.¹² However, some serious side effects, including neutropenia and vasculitis, have been encountered during its use.

There is now available a synthetic form of thymopoietin suitable for clinical use. This is a pentapeptide: thymopoietin₃₂₋₃₆ (arg-lys-asn-val-thy) which retains the immunological activity of the 49 amino acid chain. This preparation has been shown to be effective in rheumatoid arthritis.¹³ So far its use has been without serious side effects, and it is to be hoped that a naturally occurring hormone might be less toxic than synthetic chemicals in the treatment of rheumatoid disease.

It was supposed that the mode of action of thymopoietin would be similar to that of levamisole in view of the similarities noted above. However, one clinical study, while showing both compounds to be effective, has revealed dissimilarities in the time course and nature of the patient's response and in the side effects produced.¹⁴

We wished to look further at the actions of these 2 drugs in chronic granulomatous inflammation and to see whether the differences apparent clinically in rheumatoid arthritis could be seen in laboratory

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models. We have therefore studied both thymopoietin and levamisole, looking for an anti-inflammatory effect in the subcutaneous carrageenan air bleb, thought to be a thymus-independent model of chronic inflammation,¹⁵ and again in the subcutaneous cotton pellet granuloma shown to be thymic-dependent.¹⁶ Thymic dependence may reflect dependence on the presence of thymic hormones themselves, the process of 'programming' T cells which takes place in the thymus, or subsequent control of T cells hormonally by the thymus.

Materials and methods

Cotton pellet granuloma. These were established according to the method of Meier *et al.*^{17 18} Dental rolls (Johnson and Johnson no. 2) were sliced to give pellets weighing 40 mg (± 1 mg). These were placed in sealed jars and autoclaved to be stored sterile until use. Male Wistar rats weighing 200–250 g were anaesthetised with ether, their backs swabbed with alcohol, and all instruments sterilised by flaming before use. Prior to implantation the cotton pellets were injected with either thymopoietin pentapeptide 0.5 ml of 1 mg/ml solution in normal saline (Janssen Pharmaceuticals), levamisole (Janssen Pharmaceuticals) 0.5 ml of 1 mg/ml solution in normal saline, or 0.5 ml normal saline as controls. Two incisions were made in the back on either side of the midline, and by blunt dissection a subcutaneous pouch was raised out into each flank. One cotton pellet was introduced into each pouch and pushed out laterally as far as possible. The incisions were each closed by two 7 mm stainless steel Michel clips. Thus each animal received 2 pellets both soaked with thymopoietin, levamisole, or saline alone.

The pellets were left in situ for 5 days, after which the animals were killed and the pellets carefully removed with the encapsulating granulomata. Each pellet was immediately weighed and then dried to constant weight (24 hours at 60°C), after which it was weighed again.

Subcutaneous carrageenan air pouch. These were prepared according to the method of Selye.¹⁹ Again male Wistar rats weighing 200–250 g were used, anaesthetised with ether, and cleaned with alcohol. All instruments were sterilised by flaming prior to use. On day 0, 20 ml of air was injected subcutaneously. Twenty-four hours later 2 ml of 2% carrageenan in saline was injected into the air pouch. From day 1 to day 9 daily injections of 0.5 ml thymopoietin pentapeptide 1 mg/ml in normal saline (Janssen Pharmaceuticals) or 0.5 ml of saline alone were given into the pouch. The levamisole treated group and their controls received oral treatment daily

with either 0.5 mg levamisole in normal saline or normal saline alone.

On the tenth day the animals were killed, the exudates within the pouches collected, and their volume and cell counts measured. A section of granuloma wall and overlying skin was taken and stained with haematoxylin and eosin. Light microscopic appearances were then studied. Comparison was then made between the thymopoietin and levamisole treated groups and their own controls.

Statistical analysis. All statistical comparisons were made by Student's *t* test.

Results

Cotton pellet granuloma. On removal at 5 days each pellet had become well encapsulated with granulomatous material and was easily dissected out from surrounding subcutaneous tissue. The granuloma in the thymopoietin treated group was smaller and less vascular than its corresponding control. The levamisole treated group showed no differences from controls on inspection alone.

Mean wet and dry pellet granulomata weights are shown in Table 1. Mean wet weight in the thymopoietin treated group was 0.4483 (± 0.1015) g and in the corresponding control group 0.5714 (± 0.1568) g. Statistical comparison showed a highly significant reduction after thymopoietin treatment ($t=6.59$; $p<0.001$). Mean dry weights in the thymopoietin treated and control groups were 0.0439 (± 0.0021) g and 0.0643 (± 0.0027) g respectively, again showing a highly significant reduction in the thymopoietin treated group ($t=5.94$; $p<0.001$). These results represent 21.66% and 45.4% reductions in wet and dry weights respectively.

The mean wet weight of the levamisole treated group was 0.8196 (± 0.1670) g and of its control group 0.6710 (± 0.1770) g, a significant increase in the levamisole treated group ($t=2.44$; $p<0.05$). Mean dry weights were 0.1752 (± 0.0442) g and 0.1273 (± 0.0412) g in the levamisole treated and control groups respectively. Statistical comparison again showed a significant increase in the levamisole treated group ($t=3.08$; $p<0.01$). These results represent 22.16% and 37.63% increases in wet and dry weights respectively after levamisole treatment.

Table 1 Mean wet and dry weights of cotton pellets after removal

Group	Mean wet weight (g)	Mean dry weight (g)
Thymopoietin*	0.4483 (± 0.1015)	0.0439 (± 0.0021)
Control	0.5714 (± 0.1568)	0.0643 (± 0.0027)
Levamisole†	0.8196 (± 0.1670)	0.1752 (± 0.0442)
Control	0.6710 (± 0.1770)	0.1273 (± 0.0412)

*Sixteen animals per group. †Eight animals per group.

Table 2 *Mean exudate volumes and cell counts from the subcutaneous carrageenan air pouch*

Group	Mean		Mean total cell count/cavity
	Exudate	Volume (ml)	
Thymopoietin*	33.1	(± 5.7)	16.3 (± 3.7)
Control	32.0	(± 2.8)	15.3 (± 2.9)
Levamisole†	25.0	(± 5.5)	32.0 (± 7.0)
Control	26.5	(± 7.2)	32.0 (± 9.8)

*Seven animals per group. †Eight animals per group.

Subcutaneous carrageenan air pouch. After 10 days examination of the pouches showed well formed cavities lined with a wall of granulomatous material and containing straw coloured exudate. Histological sections through the cavity wall and overlying skin confirmed the chronic granulomatous nature of the lesion. A single lining layer of cells was seen lying on a zone of connective tissue heavily infiltrated with mononuclear cells. No significant differences in light microscopic appearances were noted between thymopoietin and levamisole treated animals and controls.

Mean exudate volumes and cell counts are shown in Table 2. The thymopoietin treated group had a mean exudate volume of 33.1 (5.7) ml and their controls of 32.0 (2.8) ml. Mean total cell counts per cavity were 16.3 (± 3.7) and 15.3 (± 2.9) respectively. Statistical comparison showed no significant differences between groups. The levamisole treated group had a mean exudate volume of 25.0 (± 5.5) ml and its control of 26.5 (± 7.2) ml. Mean total cell counts per cavity were 32.0 (± 7.0) and 32.0 (± 9.8) respectively. Again, statistical comparison showed no significant differences between the 2 groups.

Discussion

This work has shown that neither thymopoietin nor levamisole has a significant effect on the subcutaneous carrageenan air pouch but that they have opposing effects on the subcutaneous cotton pellet granuloma. Thymopoietin significantly inhibits granuloma formation and levamisole significantly enhances it.

The carrageenan granuloma has been described as 'low-turnover'²⁰ with respect to macrophage activity and has been shown to be unaffected by thymectomy.¹³ The same work showed only slight effects on some other types of inflammation, a result consistent with the view that many features of granuloma formation are due to the presence and persistence of irritant, in this case carrageenan. Since this model is independent of thymic activity, any effects of thymopoietin or levamisole seen in our experiment could have been explained as purely anti-

inflammatory without any involvement of T lymphocytes or an immune mechanism. However, this was not the case, since neither thymopoietin nor levamisole had significant effect on this model.

In contrast, both partial and complete neonatal thymectomy have been shown to cause a striking reduction in the formation of cotton pellet granuloma.^{15 21} Adult thymectomy causes a great reduction even only 3 days later when immune deficiency is not otherwise detectable.¹⁶ From this it seems that the thymus is necessary for full expression of chronic inflammation in the cotton pellet model, where possible hypersensitivity is a factor. The effect of thymopoietin was also to cause reduction in granuloma formation in this model. This is in keeping with observations that purified thymic extract suppresses immune responses.²² Clearly therefore the thymus is closely involved in the control of chronic inflammation of the cotton pellet granuloma type. This may be either via a direct effect of thymic hormones, of which thymopoietin is one, or due to T cell function, which remains under the control of the thymus and may be dependent itself on thymic hormonal control. The first of these possibilities is unlikely, since thymectomy and the administration of thymopoietin have both produced similar results, though it is now becoming clear that a number of thymic hormones are secreted, probably having variable and sometimes opposing actions.²³ Levamisole stimulated the formation of cotton pellet granuloma. This may have been mediated via an effect on the thymus or T lymphocytes or by a direct effect on the granuloma. Evidence in support of an effect on the T lymphocytes comes from work showing that levamisole increases circulating T lymphocytes in deficient individuals with rheumatoid arthritis²⁴ and experimental animals,²⁵ an effect which it shares with thymopoietin. However, levamisole has been considered to be an immunostimulant, as shown by its effect, in common with penicillamine, on delayed hypersensitivity.^{26 27} Indeed it was for this reason that a possible beneficial effect in rheumatoid arthritis was postulated²⁸ and the drug introduced for the treatment of this disease.¹²

Thus there is evidence that, while levamisole stimulates some forms of chronic inflammation and some immunological mechanisms, thymopoietin suppresses them. Undoubtedly the profile of activity of these drugs overlaps in some respects, particularly in the effect on peripheral T cell differentiation. However, neither this laboratory work nor our clinical studies support the suggestion that the mode of action of these 2 agents is the same.

Neither levamisole nor thymopoietin are at present available on prescription and can be obtained only from Janssen Pharmaceuticals, Beerse, Belgium.

References

- 1 Thienpont D, Vanparijs O F J, Raeymaekers A H M, *et al.* (R8299) A new potent broad spectrum antihelminthic. *Nature* 1966; **209**: 1082-4.
- 2 Symoens J, Rosenthal M. Levamisole in the modulation of the immune response. The current experimental and clinical state. *J Reticuloendothel Soc* 1977; **21**: 175-221.
- 3 Goldstein G. Isolation of a bovine thymin. A polypeptide hormone of the thymus. *Nature* 1974; **247**: 11-4.
- 4 Schlesinger D H, Goldstein G. The amino acid sequence of thymopoietin 11. *Cell* 1975; **5**: 361-5.
- 5 Scheid M P, Goldstein G, Boyse E A. The generation and regulation of lymphocyte populations. Evidence from differentiative induction systems in vitro. *J Exp Med* 1978; **147**: 1727-43.
- 6 Lau C Y, Goldstein G. Functional effects of thymopoietin 32-36 (TP₃) on cytotoxic lymphocyte precursor units. *J Immunol* 1980; **4**: 1861-5.
- 7 Hardy M A, Goldstein G, White A. Immunological effects of purified thymic hormone thymosin on neonatally thymectomised mouse. *Surg Forum* 1972; **23**: 305-7.
- 8 Laghi Pasini F, Auteri A, Pasqui A L, Di Perri T. Immunokinetics of a single dose of thymopoietin pentapeptide. *Int J Immunopharmacol* 1980; **2-3**: 154-5.
- 9 Wara D W, Goldstein G, Doyle N E, Ammann A J. Thymosin activity in patients with cellular immunodeficiency. *N Engl J Med* 1975; **292**: 70-4.
- 10 Scheinberg M A, Blacklow N R, Goldstein A L, Parrino T A, Rose F B, Cathcart E S. Influenza: response of T cell lymphopenia to thymosin. *N Engl J Med* 1976; **294**: 1208-11.
- 11 Verhaegen H, De Cree J, De Cock W, Verbruggen F. Restoration by levamisole of low E rosette forming cells in patients suffering from various diseases. *Clin Exp Immunol* 1977; **27**: 313-8.
- 12 Huskisson E C, Dieppe P A, Scott J, Trapnell D, Balme H W, Willoughby D A. Immunostimulant therapy with levamisole in rheumatoid arthritis. *Lancet* 1976; **i**: 393-5.
- 13 Veys E M, Huskisson E C, Rosenthal M, *et al.* Clinical response to therapy with thymopoietin pentapeptide (TP₅) in rheumatoid arthritis. *Ann Rheum Dis* in press.
- 14 Thrower P A, Doyle D V, Scott J, Huskisson E C. Thymopoietin in rheumatoid arthritis. *Rheumatoid Rehabil* in press.
- 15 Rothwell T L W, Spector W G. The effect of neonatal and adult thymectomy on the inflammatory response. *J Pathol* 1972; **108**: 15-21.
- 16 Girerd R J, Di Pasquale G. Thymus and inflammation. *J Endocrinol* 1963; **26**: 209-13.
- 17 Meier R, Schuler W, Desaulles P. Zer Frage des Mechanismus de Hemming des Bindegewebswahrstums zur Cortisone. 1950; *Experientia* **6**: 469-71.
- 18 Meyer R K, Stucki J C, Aulesbrook K A. Effect of pregnancy and lactation on granuloma tissue formation and joint permeability in rats. *Proc Soc Exp Biol Med* 1953; **84**: 624-8.
- 19 Selye H. Use of granuloma pouch technique in the study of antiphlogistic corticoids. *Proc Soc Exp Biol N Y* 1953; **82**: 328-33.
- 20 Ryan G B, Spector W G. Natural selection of long-lived macrophages in experimental granulomata. *J Pathol* 1969; **99**: 139-51.
- 21 Heilmeyer L, Kasemir H, Derp L. Thymus and inflammation. *German Med Monthly* 1968; **13**: 441-2.
- 22 Bach J F, Bach M A, Charreire J, Dardenne M, Erard D, Riveau-Kaiserlian D. The effect on serum thymic factor on suppressor T cells. In: Hadden J, Chedid L, Mullen P, Spreafico F, eds. *Advances in Immunopharmacology*. Oxford: Pergamon Press, 1981.
- 23 Low T L K, Thurman G B, Zatz M M, Hu S K, Goldstein A L. A multifaceted role for thymosin and its composite peptides in T cell regulation. In: Hadden J, Chedid L, Mullen P, Spreafico F, eds. *Advances in Immunopharmacology*. Oxford: Pergamon Press, 1981.
- 24 Rosenthal M, Trabert U, Müller W. The effect of levamisole on peripheral blood lymphocyte subpopulations in patients with rheumatoid arthritis and ankylosing spondylitis. *Clin Exp Immunol* 1976; **25**: 493-6.
- 25 Van Ginckel R F, Hoebeke J. Effects of levamisole on spontaneous rosette forming cells in murine spleen. *Eur J Immunol* 1976; **6**: 305-7.
- 26 Arrigoni-Martelli E, Bramm E, Huskisson E C, Willoughby D A, Dieppe P A. Pertussis vaccine oedema: an experimental model for the action of penicillamine-like drugs. *Agents Actions* 1976; **6**: 613-7.
- 27 Dieppe P A, Willoughby D A, Huskisson E C, Arrigoni-Martelli E. Pertussis vaccine pleurisy: a model of delayed hypersensitivity. *Agents Actions* 1976; **6**: 618-21.
- 28 Schuermans Y. Levamisole in rheumatoid arthritis. *Lancet* 1975; **i**: 151.