Thymopentin treatment in severe atopic dermatitis—clinical and immunological evaluations

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Abstract
An open clinical trial of thymopentin was conducted on 12 children with severe atopic dermatitis. The patients were treated with injections three times a week of 50 mg thymopentin for six weeks. They were then divided randomly into two groups: group A continued thymopentin for an additional six weeks, and group B were treated with normal saline. Clinical parameters and immunological function were evaluated serially. The total severity score started to decline from baseline significantly three weeks after treatment, and continued throughout the study period in group A but began to flare up in group B two weeks after stopping thymopentin. All the eight patients in group A completed the trial but three out of eight in group B dropped out because of flaring up of skin lesion. In vitro production of interleukin-4 tended to decrease and that of interferon gamma tended to increase, but total serum IgE, in vitro IgE synthesis, and abnormally low CD8+ CD11b+ suppressor T cell remained unchanged. Histamine releasing factor (HRF), plasma histamine, and respiratory burst activities of polymorphonuclear leucocytes were appreciably decreased after thymopentin treatment.

It is concluded that the clinical efficacy of short term thymopentin treatment very possibly results from the decreased production of HRF and decreased release of polymorphonuclear leucocyte derived inflammatory mediators and may have no relation with antigen-IgE immune reaction.

Atopic dermatitis is a chronic cutaneous inflammatory disease characterised by early age of onset, severe pruritus, typical morphology and distribution of skin rash, chronic relapsing course, and personal or family history of atopy. It is a common disease among infants and children with an incidence of 1-9% to 8.3% in white people and 1-24% in Chinese schoolchildren. Chronic atopic dermatitis may result in significant morbidity, including hospitalisation for control of skin disease and infection, lost school days, psychological trauma from physical disfigurement, and occupational disability. The management of atopic dermatitis has been less than satisfactory. None of the currently available treatments are curative and treatment is empirical. Although the pathogenesis of atopic dermatitis remains unclear, a number of immunological abnormalities have been described. They include:

1. Raised and sustained serum IgE concentrations,
2. Increased natural killer cell activity,
3. Defective capacity to generate alloreactive cytotoxic T cells,
4. Impaired autologous mixed lymphocyte reaction due to defective CD4 responder T cells,
5. Increased cell mediated cytotoxicity against skin fibroblasts,
6. Most importantly, immunological reconstitution after bone marrow transplantation results in the permanent resolution of eczema in Wiskott-Aldrich syndrome, a primary T cell immunodeficiency with raised serum IgE, providing in vivo evidence that immune defects predispose to the development of eczema.

Thymopoenin is a polypeptide hormone of the thymus, originally isolated from bovine thymus extract by its effect on neuromuscular transmission. It is secreted by thymic epithelial cells and has been shown to influence both the differentiation of thymocytes and the function of mature T cells. Thymopentin (Timunoxy, Immunobiology Research Institute, Annadale, NJ) is the active pentapeptide (Arg-Lys-Asp-Val-Tyr) moieties corresponding to amino acids 32 to 36 of the linear 49 amino acid sequence of thymopoietin. The biological effects of thymopentin mimic those of thymopoietin in all systems in which it has been evaluated. It was marketed in Italy in 1985, in Belgium in 1987, and in Germany in 1988, and has been used in a number of diseases in which T cell deficiency may have a role.

Recent studies suggest that thymopentin influences IgE synthesis and it might therefore influence the course of atopic diseases in man. Recently, clinical improvement with thymopentin that produced reductions in overall severity in patients with atopic dermatitis has been reported, but the working mechanisms have not been studied extensively. We present the clinical efficacy of an open trial of thymopentin in children with severe atopic dermatitis and correlate the clinical efficacy with the in vivo and in vitro immunological changes after thymopentin treatment.

Subjects and methods

Subjects
Sixteen children, nine boys and seven girls, aged 3 to 14 years, were enrolled into this study.
The clinical trial was approved by the human research committee of this hospital and informed consent was obtained for each patient. All of the patients had severe pruritus, skin lesions of characteristic morphology and distribution, chronic relapsing course, and a history of atopy, which met the criteria for the diagnosis of atopic dermatitis.1 2 The age of onset ranged from 1 month to 1 year, the mean (SD) duration of atopic dermatitis was 8.6 (3.0) years, the serum IgE concentrations ranged from 224 to 5241 IU/ml (mean (SD) 2318 (1525) IU/ml), and the mean (SD) area of involved body surface was 81.5 (25.0)%.

The protocol, and adverse reactions were observed carefully. Parents' satisfaction was recorded.

TREATMENT PROTOCOL
After enrolment the patients received three subcutaneous injections each week of 50 mg of thymopentin for a consecutive six weeks. The patients were then divided randomly into two groups. One group (group A) continued the thymopentin treatment for an additional six weeks, and the other group (group B) was injected with normal saline. Both groups were comparable regarding age, severity score, and serum IgE concentration. During the treatment patients were allowed to continue antihistamines and topical corticosteroids, but systemic corticosteroids were discontinued for at least four weeks before the study. No recommendation on food restriction and environmental control was made.

EVALUATION
The patients were seen every week by one author (KHH). Dermatological assessment, routine laboratory tests (blood chemistry, haematology, and urinalysis), and immunological tests were performed before and at the end of the third, sixth, and 12th week after treatment. The clinical response was evaluated by a dermatologist who did not know the treatment protocol, and adverse reactions were observed carefully. Parents' satisfaction was recorded.

IMMUNOLOGICAL TESTS
Enumeration of lymphocyte subpopulation
T cell subsets were enumerated by FACscan (Becton-Dickinson), using the monoclonal antibodies shown in Table 1.

Preparations of sera and culture supernatants
Peripheral blood mononuclear cells (MNCs) were obtained by Ficoll/Hypaque gradient density centrifugation. MNCs at a concentration of 2×10^6 cells/ml in complete culture medium (Roswell Park Memorial Institute-1640 supplemented with 10% heat inactivated fetal calf serum, antibiotics and l glutamine) were stimulated with 2 μg/ml phytohaemagglutinin (PHA) (Wellcome) for three days and the supernatants were collected by centrifugation and stored at −20°C until testing.24 25 The above mentioned culture conditions, including cell density, PHA concentration, and days of cultivation, were found to be optimal for cytokine production in our previous studies.24-26

Measurements of soluble interleukin (IL)-2R, CD4 moleculcule, and cytokines in supernatants
Cell free CD4 and IL-2 receptor tests kits were purchased from T Cell Sciences (Cambridge, MA), IL-2 and interferon gamma test kits from Genzyme (Boston, MA), and IL-4 and IL-6 test kits from R and D System (Minneapolis, MN). The principle of those tests was sandwich enzyme immunoassay (ELISA), using two monoclonal antibodies against different epitopes of the target molecule (CD4, IL-2, and IL-2R) or monoclonal antibody (first) followed by polyclonal antibody (IL-4, IL-6, and interferon gamma). The sensitivity of the ELISA was 50 pg/ml for IL-2, 50 U/ml for IL-2R, 12 U/ml for CD4, 3 pg/ml for IL-4, 3.5 pg/ml for IL-6, and 100 pg/ml for interferon gamma. The interassay variation of all tests were around 5–10% and a similar figure was found for intra-assay. All the samples of a single individual were run in duplicate simultaneously and at least one normal control was included in each experiment.

Measurement of histamine releasing factor (HRF) activity
HRF was measured as previously described.27 MNCs (2×10^6 cells/ml) were stimulated with 2 μg/ml PHA for four hours, washed, and incubated for additional 20 hours. The supernatant was collected, concentrated 50-fold, using a YM-5 Amicon membrane. The HRF activity of the supernatant was measured by its capacity to release histamine from basophils obtained from a healthy high HRF responder and was expressed as percent of total cellular histamine released. The histamine was determined by using histamine radioimmunoassay kits (Immunotech, France). The sensitivity of the kit was 0.5 nmol/l.
Measurement of serum IgG, IgA, IgM, and IgE

The IgG, IgA, and IgM were measured by nephelometry (Beckman). In vitro production of IgE was done according to the method of Saryan et al.\textsuperscript{18} The IgE concentrations in sera and culture supernatants were measured by Phadebas IgE PRIST kits (Pharmacia).

Lucigenin amplified chemiluminescence assay

Chemiluminescence was assayed by the method of Gyllenhammar,\textsuperscript{23} using a luminometer (model 1251, LKB, Bromma) and recorded as mV. Reaction mixtures contained $1 \times 10^5$ neutrophils, 0.1 mg lucigenin, and 40 ng phosphol myristate acetate (PMA) or 1 mg opsonised zymosan; and Hanks’ balanced salt solution (HBSS) was added up to a final volume of 1 ml in each reaction. The readings were started two minutes after the addition of reagents and were monitored every eight minutes up to an hour.

MEASUREMENTS OF SUPEROXIDE AND HYDROGEN PEROXIDE PRODUCTIONS

Superoxide production was measured as superoxide dismutase inhibitable reduction of ferricytochrome c with PMA or opsonised zymosan as stimulant.\textsuperscript{30} Quantitation of hydrogen peroxide is based on the horseradish peroxidase dependent oxidation of phenol red with PMA or opsonised zymosan as stimulant.\textsuperscript{31} For the superoxide assay the reaction mixture in each well consisted of ferricytochrome c (2 mg/ml), PMA (40 ng/ml), or opsonised zymosan (1 mg/ml) with neutrophils in the presence or absence of superoxide dismutase (300 U/ml). The microtitre plates were incubated and shaken at 37°C for one hour and absorbance was read at 550 nm on a Microelisa reader (MR 700, Dynatech Laboratories). For the hydrogen peroxide assay the reaction mixture in each well consisted of HBSS supplemented with 0.56 mM phenol red, 19 U/ml horseradish peroxidase, PMA (40 ng/ml) or opsonised zymosan (1 mg/ml), and neutrophils. The microtitre plates were incubated and shaken at 37°C for one hour and terminated with 10 μl of 1N sodium hydroxide. The absorbance was read at 600 nm.

Conversion of absorbance to nanomoles of both superoxide and hydrogen peroxide was calculated by a standard method.\textsuperscript{32} The results were expressed as nmol of superoxide or hydrogen peroxide/60 minutes/mg of protein.

STATISTICS

All the data were expressed as mean (SEM) except T cell subsets which was expressed as mean (SD). Unpaired Student’s t test was used for statistical analysis throughout the study except that the Wilcoxon signed rank test was used to analyse the immunoglobulin data. A p value of <0.05 was considered significant.

Results

CLINICAL RESPONSE

All eight patients in group A who received 12 weeks of continuous thymopentin injections completed the clinical trial. Three out of eight patients in group B who received thymopentin injections for the first six weeks and then switched to normal saline injections during the later half of clinical trial, however, dropped out because of flare up of skin lesions to a degree comparable with the period before thymopentin four weeks after stopping thymopentin. No difference in the total severity score was observed between two groups before and during thymopentin treatment, but the total severity score of group B was much higher than that of group A at 12 weeks ($p<0.0001$, fig 1). In both groups a therapeutic effect became evident after one week of treatment ($p<0.03$). The results of evaluation by parents matched very well with that of the physician (data not shown).

LABORATORY CHANGES

Figure 2 demonstrates, as an example, that in case 6 the maculopapulovesicles and lichenification over the neck and antecubital areas (left panel) were much improved after three weeks of thymopentin treatment (right panel). In general, erythema and pruritus were the parameters that were improved most quickly and noticeably among the five clinical parameters evaluated, whereas the improvement in scaling/dryness was much less impressive.

Figure 3 shows the in vitro productions of soluble CD4, IL-2R, and cytokines during the course of treatment. The patients produced a greater amount of soluble CD4 ($p<0.001$), IL-2R ($p<0.01$), IL-2 ($p<0.001$), and IL-4 ($p<0.001$), but less interferon gamma ($p<0.01$) than normal controls. After treatment, in vitro production of soluble CD4, IL-2R, IL-2, and IL-4 tended to decrease, but only the decrease in IL-2 ($p<0.01$) and IL-4 ($p<0.01$) reached a
Figure 2. The papulovesicles and lichenification of diseased skin before treatment (left panel) and after three weeks of treatment (right panel).

Figure 3. In vitro productions of soluble CD4, IL-2R, and cytokines during thymopentin treatment. For CD4, ***p<0.001; for IL-2, ***p<0.001, **p<0.01; for IL-2R, **p<0.01; for IL-4, ***p<0.001, **p<0.01; for IFN-γ, ***p<0.01, p<0.05. Data are expressed as mean (SEM).
significant degree. In vitro production of IFN-γ was increased (p<0.05). No change in IL-6 production was found. Again, those changes were reversed after stopping treatment (group B).

The effect of thymopentin on the distributions of T cell subsets is shown in table 2. When compared with normal controls, the patients with atopic dermatitis had a normal number (%) of total T cells (CD3+), B cells (CD19+), natural killer cells (CD3− CD16+CD56+), suppressor inducer T cells (CD4+CD45RA+), helper inducer T cells (CD4+CD45RA−), cytotoxic T cells (CD8+CD11b−), CD25+, and HLA-DR+ activated CD3 and CD4 cells. However, suppressor T cells (CD8+CD11b+) and CD25+ and HLA-DR+ activated suppressor/cytotoxic T cells (CD8+) were appreciably decreased in patients. After thymopentin treatment the suppressor T cells (CD8+CD11b+) and CD25+ activated CD8+ cells remained very low even at the end of 12 weeks of thymopentin treatment (table 2).

The changes of serum immunoglobulin concentrations were followed up serially after thymopentin treatment. IgG and IgM tended to decrease after six weeks of treatment, but not to a significant degree. No change was found for IgA, IgE, and in vitro IgE production throughout the course of trial (table 3).

Figure 4 demonstrates that both plasma histamine and HRF activity were much higher in patients than in normal controls (p<0.001 for the former and p<0.001 for the latter). After thymopentin treatment, both plasma histamine and HRF were decreased significantly. The decrease persisted in group A but reversed to pretreatment value in group at the 12th week (A ≠ B, p<0.01).

The respiratory burst activities of polymorphonuclear leucocytes after stimulation with PMA are shown in fig 5. The production of superoxide (p<0.001) and hydrogen peroxide (p<0.001) before thymopentin treatment was much higher than normal controls and the

Table 2 Changes of distributions of T cell subsets during thymopentin treatment. Results are mean (SD)%

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Treatment period (n=10)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 3 Weeks 6 Weeks</td>
<td></td>
</tr>
<tr>
<td>CD3+</td>
<td>68 (8-7) 66 (8-4) 68 (6-3)</td>
<td>67 (8-2)</td>
</tr>
<tr>
<td>CD19+</td>
<td>14 (4-8) 17 (4-9) 16 (7-0)</td>
<td>16 (4-9)</td>
</tr>
<tr>
<td>CD3−CD16+CD56+</td>
<td>17 (9-0) 17 (7-5) 16 (5-3)</td>
<td>18 (3-9)</td>
</tr>
<tr>
<td>CD4+</td>
<td>31 (9-4) 32 (0-1) 33 (5-5)</td>
<td>34 (8-1)</td>
</tr>
<tr>
<td>CD4−CD45RA+</td>
<td>10 (7-6) 10 (8-3) 11 (3-0)</td>
<td>11 (9-19)</td>
</tr>
<tr>
<td>CD4−CD45RA−</td>
<td>24 (7-5) 25 (4-6) 24 (6-6)</td>
<td>26 (7-3)</td>
</tr>
<tr>
<td>CD8+</td>
<td>28 (5-6) 26 (3-5) 29 (2-9)</td>
<td>26 (7-9)</td>
</tr>
<tr>
<td>CD8−CD11b+</td>
<td>5 (9-2-4) 3-5 (2-1) 5-6 (2-7)</td>
<td>11 (3-49)</td>
</tr>
<tr>
<td>CD8−CD11b−</td>
<td>24 (5-2) 24 (8-8) 26 (5-7)</td>
<td>20-0 (9-9)</td>
</tr>
<tr>
<td>CD3+CD25+</td>
<td>5 (0-5-1) 4 (5-99) 4 (1-34)</td>
<td>5 (1-28)</td>
</tr>
<tr>
<td>CD8+CD25+</td>
<td>5-2 (2-9) 5-6 (2-4) 5-0 (2-5)</td>
<td>6 (2-8)</td>
</tr>
<tr>
<td>CD8−CD25+</td>
<td>0-7 (1-3)** 0-2 (0-4) 0-2 (0-4)</td>
<td>3-0 (1-9)**</td>
</tr>
<tr>
<td>CD8−CD11b+</td>
<td>8 (2-5) 8 (2-4) 15 (1-87)</td>
<td>11 (3-41)</td>
</tr>
<tr>
<td>CD8+HLA−DR+</td>
<td>3 (8-9) 6 (7-26) 7 (4-26)</td>
<td>3 (6-13)</td>
</tr>
<tr>
<td>CD8+HLA−DR−</td>
<td>4-5 (3-0)** 7-8 (3-5) 8-5 (4-8)</td>
<td>10-3 (4-5)**</td>
</tr>
</tbody>
</table>

*p<0.0001, **p<0.005, ***p<0.002.

Table 3 Serum immunoglobulins and in vitro IgE synthesis during thymopentin treatment. Results are mean (SD)

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Treatment period (n=10)</th>
<th>0 3 Weeks 6 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (g/l)</td>
<td>10-24 (3-43)** 9-91 (3-32) 8-28 (1-37)</td>
<td></td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>1-52 (0-60) 1-52 (0-56) 1-48 (0-38)</td>
<td></td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>1-49 (0-72) 1-49 (0-74) 1-19 (0-59)</td>
<td></td>
</tr>
<tr>
<td>IgE(µIU/ml)</td>
<td>3283 (21666) 2862 (1563) 3026 (8109)</td>
<td></td>
</tr>
<tr>
<td>In vitro IgE synthesis (pg/ml)</td>
<td>1714 (53004 2) 2116 (42578 3) 1335 (41047)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 (Wilcoxon signed rank test).

Figure 4 Plasma histamine and HRF activity during thymopentin treatment. For plasma histamine, ***p<0.001, **p<0.01; for HRF activity, ***p<0.01, **p<0.01. Data are expressed as mean (SEM).
Figure 5  Respiratory burst activities of polymorphonuclear leucocytes stimulated by PMA. For chemiluminescence, ***p<0.001, ***p<0.001; for superoxide production, ***p<0.001, ***p<0.001; for hydrogen peroxide production, ***p<0.001, ***p<0.001. Data are expressed as mean (SEM).

Figure 6  Respiratory burst activities of polymorphonuclear leucocytes stimulated by serum opsonised zymosan. For superoxide production, ***p<0.001, ***p<0.001; for hydrogen peroxide production, ***p<0.001, ***p<0.001. Data are expressed as mean (SEM).

Discussion
All patients responded to thymopentin treatment. The itching, scratching, and erythema decreased appreciably three weeks after treatment (fig 1), and the improved skin texture due to subsidence of papulovesicles and lichenification usually occurred later (fig 2). The improvement continued in those patients (group A) who received thymopentin treatment throughout the 12 week period, however, the eczema flared up in all patients of group B four weeks after discontinuation of thymopentin (fig 1). The use of topical steroids and antihistamines could be reduced and no serious side effects were observed except transient pain at the injection site. The beneficial clinical response was largely similar to that reported by Kang et al.22 and Leung et al.23

The immunological mechanism(s) responsible for the clinical efficacy of thymopentin has not been well studied. Cooper et al reported that
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Thymopentin could influence the in vivo and in vitro production of atopic dermatitis MCNs. The group further described increased CD8+ (suppressor/cytotoxic) cells after six weeks of thymopentin administration. The results obtained in our study could not confirm their hypothesis: (i) total serum IgE and in vitro IgE production were not changed after treatment, and (ii) the CD8+CD11b+ suppressor T cells, but not the total number of CD8+ suppressor/cytotoxic T cells, were decreased in patients with atopic dermatitis, and thymopentin treatment failed to increase the CD8+CD11b+ suppressor and CD8+CD25+ activated T cells. Recent studies have demonstrated that peripheral blood MCNs from patients with atopic dermatitis produced increased concentrations of IL-4, a cytokine that induces IgE synthesis, and decreased concentrations of interferon gamma, a cytokine that inhibits IL-4 dependent IgE synthesis. Our results confirm those reports, and furthermore this study showed that thymopentin treatment tended to suppress the production of IL-4 and enhanced the production of interferon gamma in vitro. However, Li et al. recently reported that the total serum IgE and specific IgE antibodies were not decreased in patients with allergic rhinitis after receiving interferon gamma treatment in both periods of off season and during season. Vercelli et al. also found that anti-IL-4 and interferon gamma were unable to suppress the in vivo spontaneous IgE synthesis in patients with hyper-IgE syndrome, and no significant difference in mitogen induced productions of IL-4 and interferon gamma was detected between HIE patients and normal controls. Thus, mechanisms other than antigen-IgE immune reaction may account for the rather quick appearance of clinical benefit of short term thymopentin administration in the treatment of atopic dermatitis.

It is important to note that HRF and plasma histamine concentrations were decreased after thymopentin treatment (fig. 4). Furthermore, the augmented activation of polymorphonuclear leucocytes was also suppressed, as evidenced by the decreased respiratory burst activities (fig 5 and 6). Sampson et al. recently reported that patients with atopic dermatitis caused by food allergy produced significantly less HRF after the offending food allergen was eliminated from the diet for an extended period. Our recent study also demonstrated decreased production of and responsiveness to HRF in asthmatic patients benefited by successful immunotherapy. Thus, the decreased production of HRF and decreased release of polymorphonuclear leucocytes derived inflammatory mediators may account partly for the clinical efficacy of thymopentin treatment in patients with atopic dermatitis.

One of the shortcomings of this paper is lack of immunohistological studies of diseased skin before and after thymopentin treatment. Such studies would provide the most direct information that could be used to explain the clinical efficacy of thymopentin. Unfortunately, we are unable to obtain consents from parents to do skin biopsies. Finally as no serious side effects are encountered during the 12 week period of treatment and there is still no satisfactory management for severe atopic dermatitis, thymopentin may be used in those patients with atopic dermatitis who are refractory to traditional treatment. Studies with longer duration and larger number of patients are needed before the rationale for such kind of treatment can be justified.

The authors wish to thank Johnson and Johnson Company, Taiwan Branch, for supplying thymopentin preparations used in this study.

Maths and mumps
Given two different vaccines for the same disease, one more effective but with a higher complication rate than the other, which is it best to use? This problem is posed by the existence of two mumps vaccines, the Urabe Am 9 and the Jeryl Lynn strains. The former has an estimated protective efficacy of about 98% and the latter about 94% whereas the estimated complication rates are one in between 62 and 400 thousand for the Urabe Am 9 strain and one in between 250 and 1800 thousand for the Jeryl Lynn strain. D J Nokes and R M Anderson (Lancet 1991;338:1309–12) have applied a mathematical model to the available data in order to calculate the number of complications, from either the natural disease or the vaccine, to be expected over a period of 20 years after the introduction of a vaccination programme with either of the two vaccines. Incorporated within their calculations is an estimate of the degree of persistence of the natural disease at various levels of vaccine uptake within the population. Thus at 70% uptake the natural disease is still prevalent whereas at over 80% uptake the natural transmission of infection virtually ceases. Their model predicts that at the lower level of vaccine uptake (70%) the vaccine strain with greater efficacy and higher complication rate (Urabe Am 9) is associated with fewer complications overall as most of the complications at this level of vaccination are produced by the natural disease. On the other hand, at 80 to 90% uptake most of the complications are caused by the vaccine as the natural disease becomes uncommon, and the safer vaccine (Jeryl Lynn) is to be preferred.
So the answer is, if you can guarantee a high acceptance of the vaccine in your population use the safer vaccine, but if you can’t, use the more effective one.

If you can achieve high immunisation rates you automatically create an interesting problem in ethics. The natural disease is now rare and most complications arise from the vaccine. So why not cheat? Why not encourage everybody else to have their babies immunised but leave yours alone? It would have to be secret, of course, because, if everybody else tumbled to the same ruse your baby would finish up at high risk of the disease again. Why should we be concerned about the welfare of others? You may have a theology based explanation but non-theological ethicists would be forced to debate the matter endlessly without coming to any satisfactory conclusion.¹

ARCHIVIST