carrier to show whether or not the potential responses were generated by simple protein absorption to the membranes. These experiments yielded electrode potentials near the baseline and indicate that the potential changes obtained with the complete immunoelectrode are the result of interactions of the conjugate with its specific antibody. Further experiments were performed with the anti-DNP electrode by adding dinitroaniline (which mimics the coupled DNP) to the test solution. The antiserum to DNP was then added, and no potential changes were observed, an indication that the reaction at the electrode is of the antigen-antibody type.

Potential readings taken for a range of concentrations of antibody to DNP from 2.8 to 145.1 μg/ml yielded a standard deviation of 4.1 percent, with individual potential readings reproducible to $\pm$ 0.2 mV. The anti-DNP electrode retained its effectiveness for at least 2 months, and the observed potential changes were fully reversible. The anti-BSA electrode measured concentrations of antibody to BSA between 1.1 and 8.8 μg/ml with similar results. The standard deviation was 4.3 percent, and potential readings were reproducible to $\pm$ 0.6 mV. Both electrodes required from 6 to 16 minutes to reach steady-state potentials after the addition of antibody samples.

Thus, such electrodes could be used to measure antibody levels in body fluids. Although we have focused our attention on a model system involving the hapten dinitrophenol and its antibody, the preliminary work with the anti-BSA electrode has shown that this principle can be extended to other antigen-antibody systems when the immunogen can be coupled with a membrane carrier.

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7. Supported by grant GM 25308 from the National Institutes of Health.

21 March 1979

A Synthetic Pentapeptide with Biological Activity Characteristic of the Thymic Hormone Thymopoietin

Abstract. *The pentapeptide arginyl-lysyl-aspartyl-valyl-tyrosine, corresponding to amino acid residues 32–36 in thymopoietin, was synthesized. In vitro, this pentapeptide induced the differentiation of murine prothymocytes to thymocytes and inhibited differentiative induction of cells of the B lineage. This combination of actions is presently unique to the parent molecule thymopoietin. In vivo, the pentapeptide reduced the high numbers of autologous rosette-forming cells normally present in the spleens of athymic mice; this also is a property of thymopoietin. These results suggest that this readily synthesized pentapeptide corresponds to an active site of thymopoietin and might serve as a therapeutic substitute for thymopoietin.*

Thymopoietin is a thymic hormone, and the complete sequence of the 49 amino acids of this single polypeptide chain is known (*1*). Investigation of the active site or sites of this molecule has practical as well as theoretical value. For clinical use, the yield of thymopoietin from natural sources would be insufficient (*2*), it might be prohibitively antigenic, and synthesis of the entire molecule, although this has been achieved (*3*), would be impractical on a sufficiently large scale. A tridecapeptide of thymopoietin, consisting of amino acids 24 to 41 (thymopoietin$_{24-41}$), has been synthesized (*4*) and shown to share biological properties of the native molecule (*4*). We report here the synthesis of a pentapeptide that corresponds to amino acids 32 to 36 of thymopoietin, thymopoietin$_{32-36}$, (TP5), and show that it also has biological properties characteristic of thymopoietin.

The pentapeptide arginyl-lysyl-aspartyl-valyl-tyrosine was synthesized both by solid-phase methods and by classical solution synthesis. The products from both types of synthesis, purified by molecular sieving, ion exchange, and partition chromatography, were indistinguishable, and were greater than 99 percent pure according to thin-layer chromatography in five solvent systems and on high-pressure liquid chromatography. In vitro activity of TP5 was studied in induction assays of T cell and B cell differentiation (*5*), and also in vivo for effects on autologous rosette-forming cells (ARFC) (*6*) in the spleens of athymic *nu/nu* mice.

Induction assays in vitro have been of great value in elucidating the nature and mechanisms of some of the differentiative processes whereby lymphocytes progress from immunoincompetent precursors to immunocompetent cells (*5*, *7*, *8*). This progression is marked by selective expression of cell surface com-

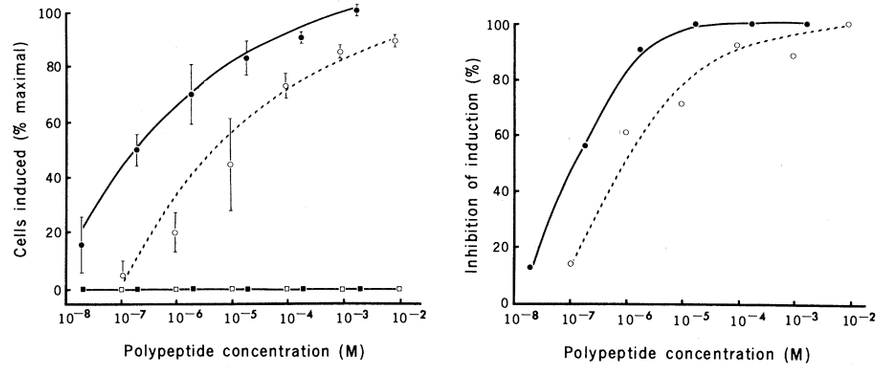

Fig. 1 (left). Selective induction of Thy-1$^+$ cells (● – ○) but not of CR$^+$ B cells (■ – □) by thymopoietin (● – ■) and TP5 (○ – □). Conditions and technique of induction of fractionated spleen cells from *nu/nu* mice were according to Scheid *et al.* (*5*). In concentrations of 10^{-6} *M* to 10^{-3}*M*, TP5 induced Thy-1$^+$ differentiation corresponding to thymopoietin over the concentration range 1.8×10^{-8}*M* to 1.8×10^{-5}*M*. To allow for variation in the proportion of inducible and induced cells in different assays, the data shown (mean $\pm$ standard error, three assays) were standardized for each assay as percent of maximal induction with optimal concentration of inducer (range, 15 to 25 percent) (*5*). Fig. 2 (right). Dose response data showing similar inhibition by thymopoietin (●) and TP5 (○) in the PC$^+$ B cell induction assay on Peyer's patch lymphocytes from *Corynebacterium parvum*–primed HSF/SN mice. Carbamyl choline (10^{-5}*M*) was used as the inducer for these experiments [induction technique of Scheid *et al.* (*5*)]. The data are standardized as percent of maximal inhibition which is complete at the higher concentrations. One of four representative experiments is shown.

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ponents. In the present study we used induction assays to monitor an early step in T cell differentiation, in which the surface component Thy-1 is acquired, and two steps in B cell differentiation: first, acquisition of complement receptors (CR), and second, the later acquisition of plasma-cell surface antigen (PC).

Thymopoietin selectively induces the differentiation of prothymocytes (Thy-1^-) to thymocytes (Thy-1^+), whereas other inducing agents induce differentiation of both prothymocytes and pro-CR^+ B cells indiscriminately (*5*). Prothymocytes and pro-CR^+ B cells are separate populations. Both are obtained from bone marrow or spleen (*5*), and both can be induced by adenosine 3′,5′-monophosphate (cyclic AMP), one of several indications that these two differentiative steps entail manifestations of predetermined programs by committed cells.

Figure 1 shows that TP5, in concentrations ranging from $10^{-6}M$ to $10^{-3}M$, has essentially the same prothymocyte-inducing capacity as thymopoietin, in a comparable range of concentrations from $1.8 \times 10^{-8}M$ to $1.8 \times 10^{-5}M$. In the CR^+ B cell induction assay, TP5 was inactive as was thymopoietin over the full range of concentrations from $10^{-7}M$ to $10^{-2}M$ (Fig. 1).

The third induction assay monitored the conversion of PC^- B cells (from Peyer's patches of mice primed with *Corynebacterium parvum*) into PC^+ B cells (*5*), presumably representing the terminal step in B cell differentiation. This third assay has given further information about the likely physiological role of thymopoietin which can be summarized as follows. First, $PC^- \rightarrow PC^+$ conversion is induced by guanosine 3′,5′-monophosphate (cyclic GMP) and inhibited by cyclic AMP, in direct contrast to prothymocyte and pro-CR^+ B cell inductions (*5*). Secondly, thymopoietin has shown only inductive activity in all T cell steps tested (*5, 7*) and only inhibitory activity in all B cell steps that have been tested. Thus it is a reciprocal hormonal regulator of T and B cell sets.

Figure 2 illustrates again that thymopoietin inhibits PC induction and shows, furthermore, that TP5 is similarly inhibitory. In control tests the tetrapeptide lysyl-aspartyl-valyl-tyrosine and the pentapeptide alanyl-lysyl-aspartyl-valyl-tyrosine neither inhibited PC induction nor induced prothymocytes. Additional synthetic derivatives are being tested for confirmation of specificity, but the reciprocal properties of TP5, namely induction of prothymocytes as opposed to inhibition of CR and PC inductions, speak highly for specificity of action related to the structure of thymopoietin.

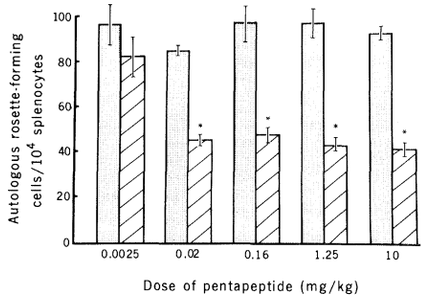

Fig. 3. The numbers of splenic autologous rosette-forming cells (ARFC) of athymic *nu/nu* mice 24 hours after intravenous injection of pentapeptide (▨) or saline (▩). Splenic ARFC were enumerated from triplicate counts of 1800 to 2000 lymphocytes as described by Van Wauwe and Van Nijen (*6*). Each bar represents the findings on four to six mice, mean ± standard error, and statistical differences (*) (Student's *t*-test) of $P < .001$ are shown.

Small numbers of thymic and splenic lymphocytes are ARFC, form rosettes with syngeneic erthyrocytes (*6, 9*), and are thought to be immature T cells. Splenic ARFC counts are high in *nu/nu* mice, and in thymectomized mice (*6, 9*) are lowered by thymopoietin in vivo (*6*); other agents, including facteur thymique serique and levamisole, have a similar effect as thymopoietin (*6*), perhaps by induction of ARFC to more differentiated T cells that lack the ARFC property. Figure 3 shows data for tests in which paired *nu/nu* littermates, matched for weight, received intravenously either TP5 in graded doses or saline alone (control). Twenty-four hours later, splenic ARFC were enumerated (*6*). Treatment with TP5 significantly reduced the number of splenic ARFC in *nu/nu* mice in doses of 0.2 to 10 mg per kilogram of body weight (Fig. 3).

In other experiments we found, as has been reported for thymopoietin (*10*), that treatment of *nu/nu* mice (Swiss, SPF, aged 4 weeks) with TP5 by daily intravenous injection yielded a 17 percent count of splenic Thy-1^+ cells as compared with the absence of Thy-1^+ cells in saline-treated controls.

According to these several criteria, TP5 shows biological activity characteristic of thymopoietin itself, and synthetic modification of such a small peptide could likely yield molecules with either enhanced activity or biologically antagonistic molecules that could block T cell differentiation. The assays that we used measure surface phenotype conversion, but additional studies with TP5 show functional effects; certain immune deficiencies of aging mice, evidently associated with thymic involution and decline in circulating thymopoietin (*11*), can be reversed by treatment with TP5 (*12*), providing further evidence that TP5 is related to the active site or sites of thymopoietin.

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14 December 1978; revised 12 February 1979