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Induction of differentiation in human marrow T cell precursors by the synthetic serum thymic factor, FTS

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SUMMARY

The serum thymic factor, 'facteur thymique serique' (FTS), was analysed *in vitro* for its ability to induce differentiation of normal human marrow T cell precursors into cells with T lymphocyte characteristics. FTS has been isolated, characterized, sequenced and synthesized. In the mouse, natural and synthetic FTS have similar activities *in vitro* in the rosette inhibition assay. Both substances influence a variety of T cell differentiation markers and functions *in vivo*. In this study, we found that synthetic FTS induced appearance of two T cell surface markers, HTLA phenotypes and the ability to form E rosettes, on a selective population of normal human marrow cells sedimenting in layers II or III of a Ficoll discontinuous density gradient. In addition, a population of lymphoid cells also found in layer III, which bears receptors for peanut agglutinin (PNA), was decreased in number following exposure to FTS. In the same gradient layer, cells which expressed terminal deoxyribonucleotidyl transferase (TdT) activity showed decreased activity after treatment with FTS. Functional activities characteristic of T lymphocytes were also enhanced in marrow cells of gradient layer III after preincubation with FTS. These T cell functions were demonstrated in marrow cells by their ability to respond and to stimulate allogeneic peripheral blood lymphocytes (PBL) in mixed lymphocyte reactions and by responses to phytomitogens, PHA, Con A and pokeweed. These changes were not observed in marrow cells of gradient layers I, IV and V or after incubation with an FTS analogue that lacked biological and antigenic activity in the mouse system.

INTRODUCTION

Several thymic peptides have been isolated, characterized and sequenced and at this juncture it appears that there is probably more than one biologically active thymic hormone or thymic factor. Some have been isolated from thymic tissues (Goldstein, 1974; Trainin *et al.*, 1975; Goldstein *et al.*, 1977) and others from serum (Bach *et al.*, 1977; Lacovera & Utermohlen, 1979).

The serum thymic factor, 'facteur thymique serique' (FTS), is a nonapeptide of mol. wt 847 daltons and is the smallest of the thymic peptides thus far isolated in an apparently native state (Bach *et al.*, 1977; Dardenne *et al.*, 1977). Its thymic epithelial dependence has been demonstrated (Dardenne *et al.*, 1974) and its presence in thymus has also been established by chemical and radioimmunological analyses (Bach *et al.*, 1978). An antiserum raised against synthetic FTS has been shown by immunofluorescence to bind specifically to the thymic epithelium (Monier *et al.*, 1979). FTS has been sequenced and synthesized (Pleau *et al.*, 1977) and synthetic FTS has chemical

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characteristics similar to natural FTS (Bach *et al.*, 1978). Synthetic and natural FTS have been demonstrated also to have similar activities in the rosette inhibition assay *in vitro*. Furthermore, both substances influence expression of a variety of T cell differentiation markers and functions in the mouse *in vivo* (Bach *et al.*, 1978; Dardenne, Charriere & Bach, 1978; Bach, 1977).

Studies on T cell differentiation *in vitro*, conducted first in the mouse (Bach *et al.*, 1971; Komuro & Boyse, 1973), then in man (Incefy, Touraine & Good, 1974; Touraine *et al.*, 1974; Incefy, L'Espérance & Good, 1975; Incefy *et al.*, 1976, 1978) have demonstrated that thymic extracts or purified thymic peptides can induce the expression of surface markers on T cell precursors as well as the development of functional activities that are characteristic of T lymphocytes. In this study, we have analysed the influence of the serum thymic factor, FTS, on differentiation of human T cells *in vitro* (Incefy *et al.*, 1979). We have found, further, that synthetic FTS can enhance differentiation of a selective population of normal marrow T cell precursors into cells which exhibit surface markers and functional activities of T lymphocytes. In addition, a certain population of marrow lymphoid cells that possess receptors for peanut agglutinin (PNA) decreased in number following exposure to FTS and those with terminal deoxyribonucleotidyl transferase (TdT) activity also showed a decrease of enzyme activity after treatment with FTS. These changes were not observed after incubation with an FTS analogue that lacked biological and antigenic activity in the mouse systems (Bricas *et al.*, 1977; Bach *et al.*, 1978). To our knowledge, the studies presented here represent the most complete analysis of the differentiative influence of a synthetic thymic factor thus far presented.

MATERIALS AND METHODS

Fractionation of human marrow cells. Bone marrow was obtained from healthy volunteers (24–36 years of age) by multiple small aspirations from the posterior iliac crest. Informed consent from all donors was obtained. Marrow aspirates were diluted 1/4 (v/v) with RPMI 1640 (GIBCO, Grand Island, New York) containing 50 u/ml of penicillin and 50 μg/ml of streptomycin (RPMI medium). Enrichment of T cell precursors was accomplished by fractionation on Ficoll density gradient as described (Incefy, L'Espérance & Good, 1975).

Induction procedure. Marrow cells from the original cell suspension and those from each gradient layer were resuspended to 4×10^6 cells/ml with RPMI medium containing 5% AB^+ serum (medium A). Cells from each fraction were then incubated at 37°C in medium A (controls) or with various concentrations of FTS, or the FTS analogue, for 2 or 15 hr in a humidified 5% CO_2–95% air incubator. Cells were washed after incubation and their concentration determined in a Cytograf (Ortho Instruments, Westwood, Massachusetts).

The inducing agent, FTS, as a purified factor prepared from pig serum, and the synthetic peptides, FTS, or the FTS analogue (Bach *et al.*, 1977; Bricas *et al.*, 1977) were assayed for activity in the rosette inhibition assay prior to use. No differences have been observed between natural and synthetic FTS by all approaches, chemical, biological and radioimmunoassays. The FTS analogue, used as a control in our studies, is an octapeptide with the same amino acid composition and sequence as natural FTS but it lacks the terminal amino acid, asparagine (Asn), at the carboxyl end of the FTS peptide (Bricas *et al.*, 1977). It has been reported not to have biological activity and not to be recognized by antibody to FTS (Bach *et al.*, 1978).

Surface marker analysis. Appearance of three T cell surface markers was investigated on marrow cells after incubation with FTS or its analogue. Human T lymphocyte antigenicities (HTLA) were detected in a microcytotoxicity test in the presence of a specific heterologous anti-human T cell serum and complement. The preparation of this antiserum has been described previously (Incefy *et al.*, 1975). E rosette-forming cells (E-RFC) were detected by spontaneous E rosette formation with SRBC (Incefy, L'Espérance & Good, 1975).

Fluorescein-coupled PNA (PNA-FITC) was either a generous gift of Dr R. Lotan or was purchased from Vector, Burlingame, California and used according to the procedure of London, Berrih & Bach (1978). Optimal conditions for staining human thymocytes and marrow cells were established. In brief, 0·1 ml of 4×10^6 cells/ml were mixed with 10 μl of 0·50 and 0·75 mg/ml

PNA-FITC and kept for 30 min at 4°C. Cells were washed three times by centrifugation with RPMI 1640 containing 10% foetal calf serum (FCS) and 0·02% Na azide. The final cell pellets were resuspended in 50 μl of the same medium and PNA+ cells were examined in a Leitz Ortholux II microscope equipped with epifluorescence objectives (NPL Fluotar 10/0·30 and NPL Oel 100/1·30) and eyepiece periplan GF × 12·5. Since PNA binds also to cells of the granulocyte (myelocytic) system and is ingested by phagocytizing cells, PNA+ cells were scored as PNA+ lymphoid or non-lymphoid cells. From 200 to 300 marrow cells were counted for each determination.

Enzymatic activity of terminal deoxynucleotidyl transferase (TdT). Changes in activities of TdT, as a marker of prothymocytes, were determined in each cell fraction before and following 2 hr of incubation with FTS by the procedure of Modak *et al.* (1979). The assay contained 50 μM ^{3}H-dGTP (sp. act. 1,000 c.p.m./pmol) as substrate and 50 mg/ml oligo dA as primer (Mertelsmann *et al.*, 1978). Results were calculated from the difference in incorporation observed in the absence and presence of 100 μM ATP and expressed as units/10^8 cells (1 unit = 1 nmol ^{3}H-dGMP incorporated per hr at 37°C).

Functional activities analysis. Induction of T lymphocyte functional activites, acquired by certain marrow cells during preincubation with FTS, were analysed by their proliferative responses to mitogens (PHA, concanavalin A (Con A) and pokeweed (PWM)) or to allogeneic PBL in mixed lymphocyte culture reaction (MLR). Briefly, after preincubation with FTS or its analogue for 15 hr in sterile tubes in a humidified 5% CO_2–95% air incubator, marrow cells were washed three times with medium A and concentration adjusted to 1×10^6 cells/ml before stimulation with mitogens or irradiated PBL (1,600) as described (Cunningham-Rundles, Hansen & Dupont, 1976; Dupont, Hansen & Yunis, 1976) The various mitogen concentrations listed in Figs 8, 9 and 10 represent final concentrations used (μg/ml of culture). Cells were cultured for 60 hr with mitogens or 120 hr with PBL and labelled with 1 μCi of ^{3}H-thymidine (New England Nuclear, Boston, Massachusetts) for 24 hr prior to harvesting. Each determination was performed in triplicate. Cells were collected on glass-fibre filters at the end of incubation with a Skatron cell harvester (Flow Laboratories, Rockville, Maryland) and counted as d.p.m. in a Packard liquid scintillation spectrometer (Packard Instrument, Chicago, Illinois).

RESULTS

The procedure used for *in vitro* study of T cell differentiation has already been described (Incefy,

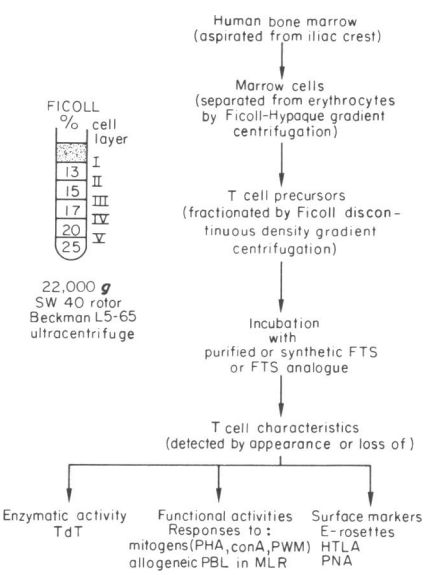

Fig. 1. Procedure for the *in vitro* study of T cell precursor differentiation under the influence of 'facteur thymique serique' (FTS) in normal human marrow.

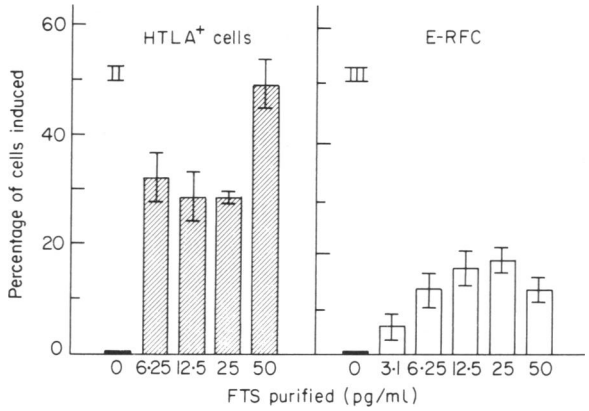

Fig. 2. Induction of T lymphocyte surface markers on T cell precursors from normal human marrow after 15 hr of incubation with purified FTS. Marrow cells were fractionated and studied for appearance of surface markers according to the procedure described in Fig. 1. Cells induced to bear HTLA phenotypes and form spontaneous E rosettes with SRBC (E-RFC) sedimented in layers II and III of the Ficoll discontinuous density gradient. The percentage of cells induced was calculated as follows: $100\ (a-b)/(100-b)$, where a = % $HTLA^+$ cells or % E-RFC after incubation with FTS or its analogue, b = % $HTLA^+$ cells or % E-RFC after incubation with medium (control). Mean ± s.d. is given.

L'Espérance & Good, 1975) and is outlined in Fig. 1. In addition to investigating the appearance of T cell surface markers after incubation with FTS, we have also analysed (a) a certain population of cells bearing receptors for the lectin, PNA, (b) changes in enzymatic activity of TdT and (c) T cell functional activities, detected by responses to mitogens or to allogeneic PBL in MLR.

Analysis of surface markers

Using this procedure, we could detect the appearance of two T cell surface markers after 15 hr of incubation with purified FTS, when used at 6·25 to 60 pg/ml. Marked increases in the number of cells bearing HTLA phenotypes were seen in marrow cells of gradient layer II while increases in receptors for SRBC (E-RFC) were detected in layer III (Fig. 2). Although a very small number of cells could also be induced to the stage of E-RFC in layer II (not shown), induction of cells bearing SRBC receptors was more pronounced in layer III and was dose-dependent, the greatest increase being observed after incubation with 25 pg/ml of FTS. Marrow cells of gradient layers I, IV and V did not show these changes after incubation with the peptide.

Similar increases in the number of cells bearing HTLA and E rosette markers (E-RFC) were observed after 15 hr of incubation with synthetic FTS, when analysed over the range 1 pg to 100 ng

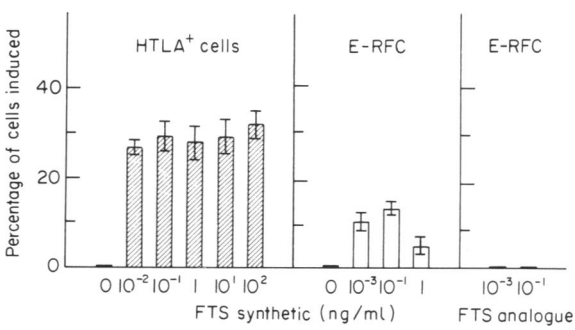

Fig. 3. Induction of T lymphocyte surface markers on T cell precursors from layer III of normal human marrow after 15 hr of incubation with synthetic FTS. Marrow cells were studied as described in Fig. 1 and in the legend to Fig. 2. Appearance of surface markers (HTLA and E-RFC) was observed on T cell precursors sedimenting in layer III of the Ficoll discontinuous density gradient. The FTS analogue, used as control, was tested under the same experimental conditions as synthetic FTS (10^{-1} and 10^{-3} ng/ml). Mean ± s.d. is given.

(i.e. 10^{-3}–10^{2} ng/ml) (Fig. 3). In this particular experiment, both surface markers were induced in significant numbers on cells of layer III and the difference from the results shown in Fig. 2 could be attributed to the use of marrow cells from a different normal donor. We have observed similar variability in the past; however, the appearance of receptors for SRBC is observed most often on cells found in a gradient layer below the one where HTLA is induced (Incefy *et al.*, 1978). The percentages of lymphoid cells, identified morphologically, induced to bear HTLA or E rosette markers by 10 pg/ml of synthetic FTS (10^{-2} ng/ml in Fig. 3) were found to be 27 and 12% respectively, whereas those induced by 12·5 pg/ml of purified FTS were 29 and 16% respectively (Fig. 2). These results demonstrate that synthetic and purified FTS have similar effects *in vitro* in their ability to induce the appearance of T lymphocyte surface markers on human marrow T cell precursors. When tested under the same experimental conditions, the FTS analogue did not produce changes of surface markers on marrow cells (Fig. 3).

Ability to bind PNA has been demonstrated to be a characteristic of certain early or immature T cell subsets, present on 70–80% of cortical thymocytes and on 5–15% marrow cells, but mostly absent from PBL (Reisner, Linker-Israeli & Sharon, 1976; Reisner *et al.*, 1978). In an attempt to characterize certain specific stages of sequential development of T lymphocytes, we analysed changes in the number of cells bearing PNA receptors among marrow cells of the various gradient layers after 15 hr of incubation with synthetic FTS. Sixty-seven per cent of marrow cells from layer III were found to be PNA^+; of those, 57% were non-lymphoid and belonged to the granulocytic or monocytic systems and 10% were lymphoid. As shown in Fig. 4, nearly 50% of those lymphoid PNA^+ cells lost the marker following incubation with FTS at 10^{-3} ng/ml. No significant changes were observed among the non-lymphoid PNA^+ cell populations of layer III following the same treatment. No change in either lymphoid or non-lymphoid cells was detected with the FTS analogue in a different experiment (data not shown).

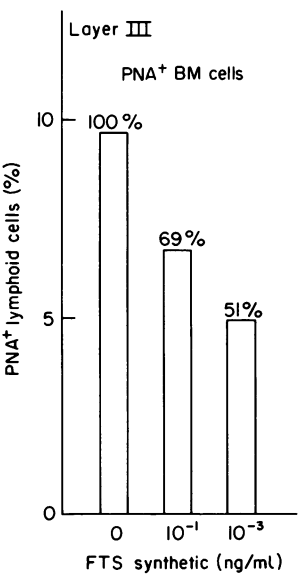

Fig. 4. Decreased number of PNA^+ lymphoid cells in normal marrow cells of Ficoll gradient layer III after 15 hr of incubation with synthetic FTS (10^{-1} and 10^{-3} ng/ml). Marrow cells were studied as described in Fig. 1. Data from a representative experiment of a total of three is presented.

Enzymatic activity of TdT

Activity of TdT was investigated before and after incubation with FTS. As already reported, TdT activity is highest in thymocytes and low in bone marrow cells (Kung *et al.*, 1975; Mertelsmann *et al.*, 1978) but is found also in prothymocytes (Silverstone *et al.*, 1976). We could detect up to 133 units of TdT activity per 10^8 cells in normal human thymocytes but only 0·032 units/10^8 cells in unfractionated normal human marrow cells before and after 2 hr of incubation in medium A

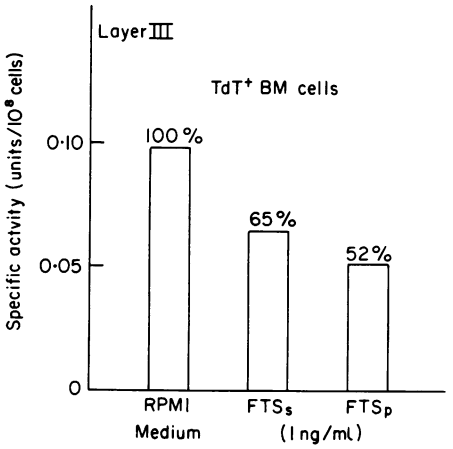

Fig. 5. Decreases in TdT specific activity in normal marrow cells of Ficoll gradient layer III after 2 hr of incubation with synthetic (s) or purified (p) FTS (1 ng/ml). Marrow cells were studied as described in Fig. 1. Specific activity is expressed as units/10^8 cells. One unit of TdT activity is equal to 1 nmol ^{3}H-dGMP incorporated per hr at 37°C. Data from a representative experiment of a total of four is presented.

respectively. After fractionation on Ficoll discontinuous density gradient, TdT activity was generally present in marrow cells of layers II and III at levels of 0·12 and 0·20 units/10^8 cells respectively. In four experiments using marrow cells from different donors, changes in enzyme activity were analysed after 2 hr of incubation with purified or synthetic FTS, FTS analogue or medium A in cells of gradient layers II, III and IV. Decreased TdT activity could only be observed reproducibly in marrow cells of layer III following incubation with FTS, as shown in a representative experiment in Fig. 5. TdT activities remaining in those cells after 2 hr of incubation with 1 ng/ml of purified or synthetic FTS were 65 and 52% of the control values, whereas activity of the enzyme did not decrease after incubation with medium A or the FTS analogue under similar conditions. In two additional experiments, smaller decreases in TdT activity were also observed in marrow cells of layer II. These observations are to be sharply distinguished from those reported earlier by Pazmino,

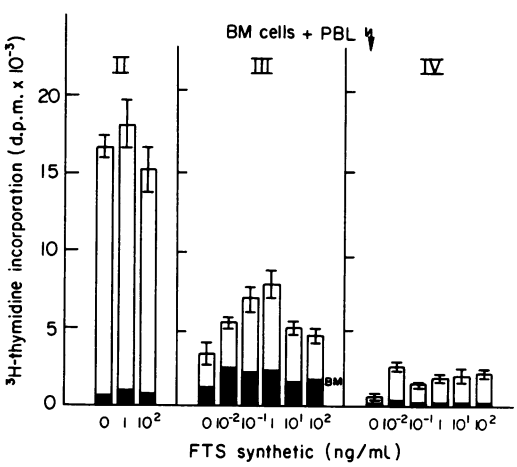

Fig. 6. Response of normal human marrow cells from Ficoll gradient layers II, III and IV in mixed lymphocyte culture reaction (MLR). Marrow cells were preincubated for 15 hr with synthetic FTS, washed and co-cultured with irradiated PBL (PBL ↯) for 120 hr. Cells were labelled for 24 hr with ^{3}H-thymidine prior to harvesting (see Materials and Methods section). Increases in ^{3}H-thymidine incorporation for marrow cells of layer III that were preincubated with FTS (10^{-1} and 1 ng/ml) are statistically higher than for those preincubated with medium only ($P < 0{\cdot}05$). Black areas at bottom of bar graph represent spontaneous DNA synthesis of bone marrow cells (BM) only; statistically high ($P < 0{\cdot}001$) in cells preincubated with FTS (10^{-2}, 10^{-1} and 1 ng/ml). Mean ± s.d. is given.

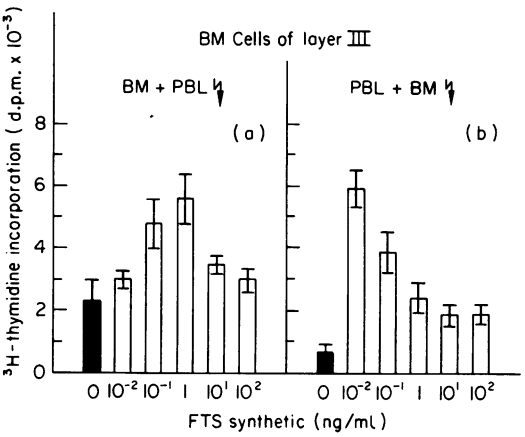

Fig. 7. Enhancement in ability of marrow cells to respond or to stimulate in MLR after 15 hr of preincubation with synthetic FTS: (a) after exposure to FTS, bone marrow cells (BM) of Ficoll gradient layer III were co-cultured for 120 hr with allogeneic PBL (BM + PBL ↯) or (b) irradiated BM were co-cultured with allogeneic PBL (PBL + BM ↯). Cells were labelled as described in the legend to Fig. 6. (a) Increases over control are statistically significant ($P < 0.05$) after preincubation with FTS at 1 ng/ml, and (b) ($P < 0.01$ to < 0.05) after preincubation with FTS at 10^{-2}, 10^{-1} and 1 ng/ml. Mean ± s.d. is given.

Ihle & Goldstein (1978) who observed increasing TdT activity in marrow cells of thymectomized or nu/nu mice after *in vivo* or *in vitro* treatment with thymosin-F5.

Analysis of functional activities

The effect of synthetic FTS on functional activities characteristic of T lymphocytes was then investigated. Marrow cells from Ficoll gradient layers II, III and IV only were studied because too few cells were available in layers I and V in most experiments. Following preincubation with synthetic FTS, responses of marrow cells to allogeneic PBL in MLR are shown in Fig. 6. Marrow cells of layer II, which included many large cells appearing by morphology to belong mostly to the

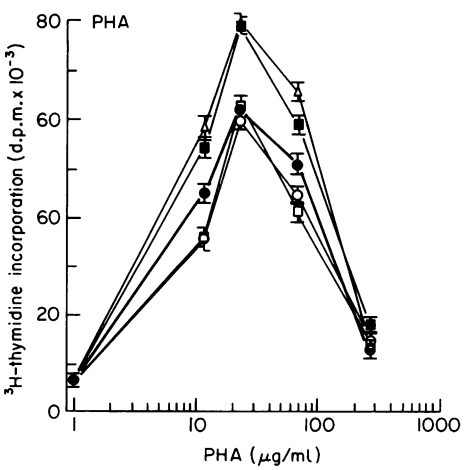

Fig. 8. Enhanced PHA responses in marrow cells of Ficoll gradient layer III after exposure to synthetic FTS. Marrow cells were preincubated for 15 hr with FTS at various concentrations: (●—●) RPMI control, (○—○) FTS_s 10^2 ng/ml, (□—□) FTS_s 10^1 ng/ml, (△—△) FTS_s 1 ng/ml, (■—■) FTS_s 10^{-2} ng/ml. Cells were cultured further with PHA at final concentrations of 11, 23, 68 or 270 μg/ml of culture for 60 hr and labelled for 24 hr with ^{3}H-thymidine prior to harvesting as described in the Materials and Methods section. Differences from control were greatest and statistically significant ($P < 0.01$) when marrow cells were preincubated for 15 hr with FTS at 10^{-2} and 1 ng/ml and cultured with PHA at 23 μg/ml. Mean ± s.d. is given.

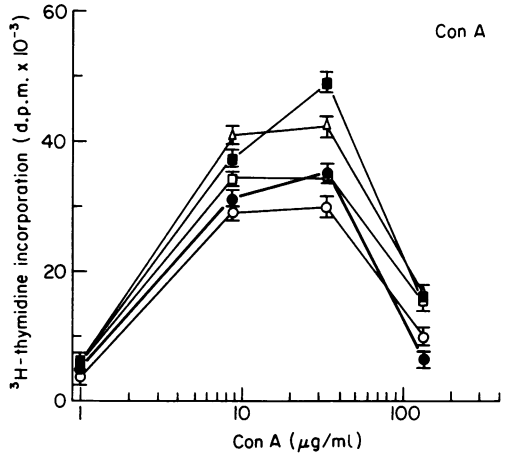

Fig. 9. Enhanced Con A responses in marrow cells of gradient layer III after exposure to synthetic FTS. Cells were preincubated with FTS as indicated in the legend to Fig. 8 and cultured further with Con A at 9, 34 or 135 μg/ml of culture for 60 hr. (●—●) RPMI control, (○—○) FTS_s 10^2 ng/ml, (□—□) FTS_s 10^1 ng/ml, (△—△) FTS_s 1 ng/ml, (■—■) FTS_s 10^{-2} ng/ml. Cells were labelled as described in the Materials and Methods section. Differences from control were greatest and statistically significant ($P < 0{\cdot}01$) after exposure to FTS at 10^{-2} and 1 ng/ml and cultured with Con A at 34 μg/ml. Mean ± s.d. is given.

myeloid series, possessed high spontaneous DNA synthesis with medium alone, whereas those of layers III and IV did not. Cells of layers II and IV showed insignificant changes after 15 hr of preincubation with synthetic FTS, whereas those of layer III responded to irradiated PBL with marked increases in DNA synthesis (statistically significant at $P < 0{\cdot}05$) after FTS exposure while control cells incubated with medium alone did not. Maximal increases were observed at 1 ng/ml of FTS. Significant increases in spontaneous DNA synthesis ($P < 0{\cdot}001$) were also observed in cells of layer III, as indicated by the black areas at the bottom of the bar graph. These changes were detected in cells preincubated for 15 hr with FTS, then cultured alone for 5 days without exposure to allogeneic PBL. When corrections were made for spontaneous cell proliferation, marked enhancement in MLR could still be detected (Fig. 7a). As shown, marrow cells of layer III were both

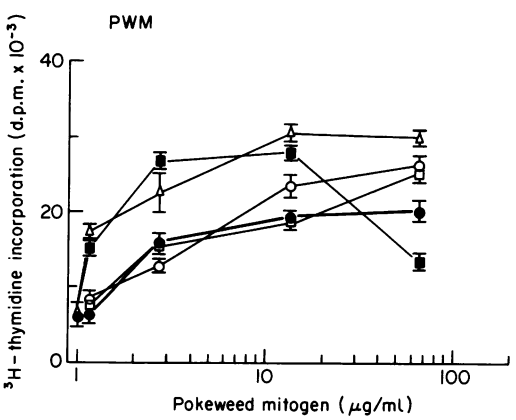

Fig. 10. Enhanced PWM responses in marrow cells of gradient layer III after exposure to synthetic FTS. Cells were preincubated with FTS and cultured with PWM at 3, 14 or 68 μg/ml of culture for 60 hr. (●—●) RPMI control, (○—○) FTS_s 10^2 ng/ml, (□—□) FTS_s 10^1 ng/ml, (△—△) FTS_s 1 ng/ml, (■—■) FTS_s 10^{-2} ng/ml. Cells were labelled as described in the Materials and Methods section. Differences from control were greatest and statistically significant ($P < 0{\cdot}01$) after preincubation with FTS at 10^{-2} and 1 ng/ml and when cultured with PWM at 3 and 14 μg/ml. Mean ± s.d. is given.

enhanced by FTS to respond more sharply to irradiated PBL ($P<0{\cdot}05$ at 1 ng/ml), and became better able to stimulate non-irradiated PBL in MLR ($P<0{\cdot}01$ to $0{\cdot}05$) (Fig. 7b).

Responses to mitogens following treatment with synthetic FTS were also analysed in marrow cells of gradient layers II, III and IV. Small, but reproducible and statistically significant increases in responses to PHA (Fig. 8), Con A (Fig. 9) and PWM (Fig. 10) were observed in marrow cells of layer III that had been preincubated for 15 hr with 1 and 10^{-2} ng/ml of FTS. Differences from controls were most marked and statistically significant ($P<0{\cdot}01$) when marrow cells were cultured with PHA at a final concentration of 23 μg/ml, Con A at 34 μg/ml and PWM at 14 μg/ml. Preincubation with higher concentrations of synthetic FTS at 10^{1} and 10^{2} ng/ml did not produce these changes and responses to mitogens were similar to those observed with controls. None of these changes were produced by the peptide analogue of FTS which differed from the native and synthetic molecule by absence of the C-terminal Asn.

DISCUSSION

A thymic factor, isolated from serum, has been characterized, sequenced and synthesized, and named 'facteur thymique serique' (FTS) (Bach *et al.*, 1977, 1978). Its presence in serum is selectively thymic epithelium-dependent (Dardenne *et al.*, 1974; Bach *et al.*, 1978; Monier *et al.*, 1979). Natural and synthetic FTS have been analysed in mouse *in vitro* and *in vivo* and found to have similar activity, namely an ability selectively to induce appearance or disappearance of certain T cell surface markers and a variety of T cell functions (Bach *et al.*, 1978). Synthetic FTS, like other thymic hormones, has potential clinical applications in man for the treatment of certain immunodeficiency diseases, e.g. Di George's syndrome, ataxia telangiectasia and IgA deficiency with low T cell numbers, or for treatment of T cell deficiency in certain chronic infections associated with T cell deficiency, leukaemias, lymphomas and autoimmune diseases. It seemed, therefore, of importance to evaluate the influence of synthetic FTS on human marrow T cell precursors *in vitro*.

Three T cell surface markers were analysed after incubation with FTS. Two of them, HTLA and E rosettes, which are present on 95–99% of unfractionated thymocytes and 60–80% of peripheral blood lymphocytes but on only a few marrow cells, have been demonstrated to be induced by thymic extracts and purified thymic peptides on normal human marrow cells of BSA or Ficoll gradient layer III (Incefy *et al.*, 1974; Incefy, L'Espérance & Good, 1975; Incefy *et al.*, 1976; Touraine *et al.*, 1974). We found that the HTLA and E rosette markers could also be induced by both highly purified and synthetic FTS on 50 and 20% of marrow lymphoid cells of layers II and III respectively. These cell precursors did not bear the markers originally.

The third marker, recognized by the lectin PNA, is a new surface marker not yet analysed in differentiation studies by thymic factors. It appears to be a valuable marker, as is the enzyme TdT, for the study of T cell development because both permit identification of certain immature T cells at a cortical thymocyte stage of maturation. In our studies, we observed that 10% of the marrow cells in Ficoll gradient layer III which possess PNA receptors were lymphoid cells, and that nearly 50% of these cells appeared to lose the marker after exposure to FTS. This finding suggests that PNA^{+} cells probably undergo a thymus-dependent maturation step *in vitro* under the influence of FTS, since PNA receptors disappear from the surface membranes of thymocytes as these cells differentiate to T lymphocytes. The changes in TdT activity we have observed also support this point. The 50% reduction in activity of this enzyme, detected in marrow cells of layer III after a 2-hr incubation with FTS, shows that the *in vitro* phenomenon occurs in man, as already reported for the mouse by Silverstone *et al.* (1976). These authors found that at least one-half of the marrow cells with TdT activity could be eliminated after exposure to thymopoietin, by treatemnt with Thy 1·2 plus complement, suggesting that cells bearing this enzyme are related to those found in the thymus. Loss of terminal transferase activity was considered in these studies to be the earliest known property of cells that undergo thymus-dependent development before final maturation of T cells to circulating lymphocytes. It seems clear, however, that the earlier appearance of TdT marker on TdT-negative cells, induced by relatively crude thymic extracts (Pazmino *et al.*, 1978) or purified thymosin-β_3 (Hu,

Low & Goldstein, 1979), may represent an even earlier step in thymic cell development under the influence of thymic hormones.

Both synthetic and purified FTS were found to produce similar changes in human T cell surface markers *in vitro*, whereas the FTS analogue did not.

Study of the effects of this thymic factor on T cell functions demonstrated further that marrow cells in layer III could both respond to and stimulate PBL better in MLR following preincubation with synthetic FTS when compared to control cells that had been preincubated with medium only. Enhanced responses to mitogens were observed, which appear to represent functional differentiation induced by FTS. Although statistically highly significant, they were slight and thus less impressive than the numerous other consequences of exposure to FTS. This, of course, may be due to the fact that several steps could be involved in the development of these functional changes, whereas the induction of surface markers may be a more direct consequence of FTS influence. Again, these changes in functional activities were not observed after preincubation with the FTS analogue, demonstrating that they were indeed induced by the serum thymic factor itself and not by a closely associated peptide, lacking only the terminal asparagine at the carboxyl end of the molecule. The induction process of these markers is still not well understood and could be a result in rearrangement of antigens in the membrane or could be due to neosynthesis of these antigens or receptors during exposure to the thymic factor.

Finally, it can be concluded from these observations that synthetic FTS is as active as purified FTS in inducing differentiation of certain human marrow cells into cells with T lymphocyte surface markers and functions.

The changes observed with PNA and TdT seemed to indicate further that the marrow cells, which sediment mostly in layer III between density 1·0655 and 1·0736, and which undergo differentiation under the influence of FTS, are probably similar to the mouse prothymocytes reported by Silverstone *et al.* (1976). Also, it would seem that the marrow T cell precursors differentiated by FTS are at a stage of differentiation very near or equal to that represented by most cortical thymocytes. Furthermore, following exposures to FTS specifically, and not to its analogue, the marrow 'prothymocytes' appear to reach a medullary thymocyte stage of differentiation, since they did not acquire full capacity to respond to allogeneic cells in MLR or to mitogens, as is observed with normal fully differentiated PBL.

In seems likely that, since these studies were conducted *in vitro*, which does not permit exposure to other differentiative influences that might be contributed by macrophages, lymphocytes or other cells, the marrow prothymocytes did not reach full maturation under the influences described.

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