

DEMONSTRATION OF GROWTH-INHIBITORY AS WELL AS GROWTH-STIMULATORY FACTORS IN MEDIUM CONDITIONED BY LUNG LAVAGE CELLS STIMULATED WITH A CHEMOTACTIC FACTOR SECRETED BY JAAGSIEKTE TUMOUR CELLS

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ABSTRACT

MYER, M. S., VERWOERD, D. W. & GARNETT, HELEN M., 1987. Demonstration of growth-inhibitory as well as growth-stimulatory factors in medium conditioned by lung lavage cells stimulated with a chemotactic factor secreted by jaagsiekte tumour cells. *Onderstepoort Journal of Veterinary Research*, 54, 123-130 (1987)

Both growth-inhibitory and growth-stimulatory factors were detected *in vitro* in medium from chemotactically stimulated cultures of lung lavage cells. The macrophage component of the lavage cells was found to produce a growth stimulatory factor that was replaced by a growth inhibitory factor following chemotactic factor stimulation.

INTRODUCTION

Current investigations into the means whereby tumour cells regulate their environment to achieve optimum growth, has centred on the role played by leukokines, a broad class of peptides ranging in size from below 0.3 kilodaltons (kDa) to more than 70 kDa, and secreted mainly by macrophages and lymphocytes (Szaniawska, Majewski, Kaminski, Noremborg, Swierz & Janik, 1985; Skimokado, Raines, Madtes, Barrett, Benditt & Ross, 1985).

The typical increase of alveolar macrophages (AMs) in the lungs of jaagsiekte sheep is initiated by a tumour-cell-derived-chemotactic factor (Myer, Verwoerd & Garnett, 1987). Bottazzi, Ghezzi, Taraboletti, Salmona, Colombo, Bonazzi, Mangioni & Mantovani (1985) characterized a similar chemotactic factor from human ovarian carcinomas, and preliminary results indicated that this chemotactic factor in turn could activate macrophages to produce another leukokine which would enhance tumour cell growth. The term "activated macrophages", according to Van Furth (1986), refers to increased functional activity, or to a new functional activity induced by a given stimulus which needs to be defined for every situation.

Szaniawska *et al.* (1985) investigated the stimulatory and inhibitory activities of conditioned medium from lung tissue culture on the growth of normal and neoplastic cells *in vitro*. A Sephadex G-75 column fraction corresponding to a molecular mass in the region between 3 and 5 kDa was found to be a potent inhibitor of tumour cell proliferation. The findings of Bottazzi *et al.* (1985) and Szaniawska *et al.* (1985) imply that tumour cell growth in certain instances can be the result of a shift in the balance of growth stimulatory over growth inhibitory leukokines. The interaction between stimulated AMs and lymphocytes might also lead to the eventual production of a tumour cell growth factor as suggested by Skimokado *et al.* (1985).

It was the purpose of this study to determine whether the synthesis of growth stimulatory factors (GFs) and growth inhibitory factors (GIFs) could be induced in various cultures of AMs (with and without other lung lavage cells being present) from the excised lungs of

normal, healthy sheep, by means of the chemotactic factor secreted by jaagsiekte tumour cells.

MATERIALS AND METHODS

Source of chemotactic factor

The chemotactic factor is contained in the supernatant of 15.4 cell cultures (Myer *et al.*, 1987) that were initially prepared by Coetzee, Els & Verwoerd (1976).

Tumour target cells for testing the effects of GFs and GIF

A 21.3 tumour cell line prepared from the lungs of a jaagsiekte sheep by Verwoerd, De Villiers & Tustin (1980) was used, as well as another cell line of calf foetal thyroid (CFTH) cells without infection [normal (N)] and permanently infected [transformed (T)] with an actively replicating retrovirus¹.

F-12 nutrient medium

Endotoxin-free F-12 nutrient medium (Ham, 1965) containing 10 % foetal calf serum, penicillin, streptomycin and mycostatin.

Tumour target cell assay

Target cells were initially seeded at approximately 4×10^3 cells/well/0.5 ml F-12 nutrient medium in tissue culture wells for 24 h in a 5 % CO₂ incubator at 37 °C before addition of GFs or GIF. After another 24 h, the cells were tested for DNA synthesis potential by pulse labelling with ³H-thymidine² at 10 µCi/well for 72 h. The assay was terminated by washing the target cells in the culture wells 3 times with phosphate buffered saline, followed by lysis of the cells with 250 µl 1 % sodium dodecyl sulphate per well. Each lysed sample was dissolved in Beckman Ready-Solve TM scintillation cocktail and the radioactivity measured in a Beckman LS 9000 Scintillation Counter.

Tumour target cells grown in F-12 nutrient medium alone (without the later addition of GFs or GIF) were included for each experiment (except for Fig. 3) and served as a baseline control.

Stimulation of alveolar macrophages with the chemotactic factor

AMs that were isolated from the broncho-alveolar lavage performed on the excised lungs from normal, healthy sheep according to the method of Myer *et al.*

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² Specific activity 21 Ci/mmol Amersham International Ltd, Amersham, England

(1987), were chemotactically stimulated with serum-free F-12 nutrient medium containing 25 % serum-free supernatant from 15.4 cells. The AMs were cultured for 24 h at 37 °C before the supernatant was collected, and any contaminating cells were removed by centrifuging at 400 × g for 10 min. This supernatant was then stored at 4 °C before being freeze-dried at a later stage prior to column fractionation. Supernatant from unstimulated AMs was also prepared in the same way.

Normal AMs were stimulated with a series of dilutions of 15.4 cell supernatant. Four replicate cultures were seeded in tissue culture wells at 5×10^5 AMs/well/0.5 ml F-12 nutrient medium for each dilution of chemotactic factor present in the 15.4 cell supernatant (2.5 %; 5 %; 10 %; 20 %; 30 %; 40 %; 50 %; 60 %; 80 % and 100 %). Four replicates were also reserved for AMs grown in chemotactic-free F-12 nutrient medium (unstimulated AMs). After culturing both the AM replicates and parallel sets of 21.3 tumour target cell replicates in tissue culture wells for 24 h in a 5 % CO₂ incubator at 37 °C, 0.5 ml of each AM supernatant was transferred to the corresponding well containing 21.3 cells. Each transferred AM supernatant was therefore diluted 1:2 on the 21.3 cells. After another 24 h, the 21.3 cells were assayed for DNA synthesis potential using the tumour target cell assay as previously described.

Stimulation of whole lung lavage cell population with chemotactic factor

Normal AMs together with the rest of the non-macrophage lavage free-cell (Lfc) fraction were chemotactically stimulated with F-12 nutrient medium containing 25 % 15.4 cell supernatant. After incubating for 24 h at 37 °C, the supernatant was collected and added as a two-fold dilution series to 21.3 tumour target cells which were subsequently assayed for DNA synthesis potential as previously described.

Stimulation of mixed AM + Lfc cultures

In a composite experiment, the whole lavage fraction from a normal, healthy sheep was utilized. The AMs were removed by adherence to a plastic substrate as described by Myer *et al.* (1987), except that the non-adherent or Lfc fraction was retained. The Lfc was centrifuged at 400 × g and the pellet resuspended in phosphate buffered saline. This was followed by another centrifugation before the pellet was finally resuspended in serum-free F-12 nutrient medium.

The Lfc's were added to the Am cultures as follows:

- Culture flask 1: AMs without chemotactic factor stimulation
- Culture flask 2: AMs with chemotactic factor stimulation
- Culture flask 3: AMs + Lfc without chemotactic factor stimulation
- Culture flask 4: AMs + Lfc with chemotactic factor stimulation

The AM and AM + Lfc cultures were established in serum-free F-12 nutrient medium prior to the addition of 25 % serum-free 15.4 cell supernatant to flasks 2 and 4. In flasks 3 and 4, the Lfc was added back to the AM culture in the same ratio as it occurred in the lavage pellet. After incubation for 24 h at 37 °C, the supernatants were collected and any contaminating cells were removed by centrifuging for 10 min at 400 × g. The supernatants were stored at 4 °C before being freeze-dried at a later stage prior to column fractionation.

Concentration and fractionation of macrophage supernatants

The macrophage supernatants stored at 4 °C were concentrated as follows:

The first supernatant from the chemotactic factor-stimulated AMs only was concentrated 25 times by reducing 70 ml to 1 ml in an Edward's High Vacuum freeze-drier and combining this sample with supernatant that had been concentrated from 50 ml down to 4 ml, using dialysis bag pervaporation. The precaution of using 2 different methods of concentrating supernatants was taken to ensure that large molecular mass species were not altered during the freeze-drying process, and that small molecular mass species were not lost during pervaporation employing a dialysis bag with 10 kDa pore size. The 2nd series of supernatants (from the 4 flasks) was concentrated 10 times using freeze-drying only. In each case, 25 ml was completely dehydrated and then resuspended in 2.5 ml of serum-free F-12 nutrient medium containing antibiotics.

In a series of separate runs, 1 ml of each concentrated supernatant was passed through a Sephadex G-75 column previously calibrated with molecular mass markers, as described by Myer *et al.* (1987), and 1 ml fractions were collected under the same conditions as before. The 19 kDa, 25.5 kDa and 45 kDa marker positions were derived from a standard curve of log molecular mass vs fraction number, while the 7 kDa and 10 kDa positions were obtained by means of extrapolations of this curve.

GF and GIF activity of column fractions using tumour target cell assay

Samples from each column run were assayed in triplicate by adding 50 µl to the relevant tissue culture well containing 21.3 target cells. The final concentration of the first chemotactic factor-stimulated AM supernatant on the 21.3 cells was therefore 25 divided by a column dilution factor of 4 (experimentally determined) and a dilution factor of 10 when added to the culture wells. When multiplied by 100 %, the equation yielded a concentration of 60 % for the samples of the first fractionated supernatant. The concentration of the 2nd series of fractionated supernatants (from the 4 flasks), derived from a similar calculation, was 24 %.

After 24 h incubation, the 21.3 cells were assayed for DNA synthesis potential using the tumour target cell assay as previously described.

Sensitivity of growth inhibitory/stimulatory factors to heating and trypsin digestion

A number of fractions were pooled for each peak of activity (either growth suppression or stimulation of the 21.3 cells) of the different macrophage conditioned media. Samples of each peak (usually 4 fractions per peak) were then heated to either 62 °C or 100 °C or exposed to trypsin³ at a concentration of 0.6 µg/800 µl sample.

Prior to heating or enzyme treatment, 0.8 ml aliquots of each peak were split into 4 vials, where the 1st contained untreated sample, the 2nd and 3rd were used for heat treatment, and the 4th for trypsin digestion. The trypsin reaction was terminated after 2 h by the addition of 200 µl of foetal calf serum that was also added to the vials subjected to heating for 2 h, and likewise to the control samples. The treated and untreated peak samples were then assayed for biological activity, using the tumour target cell assay as previously described.

DNA synthesis potential of fibroblasts and 21.3 cells treated with a dilution series of growth inhibitory/stimulatory factors

The effect of a twofold dilution series of growth inhibitory/stimulatory factors on sheep lung fibroblasts and 21.3 cells was investigated. The fibroblasts were

³ Sigma Chemical Company, St. Louis, MO, USA

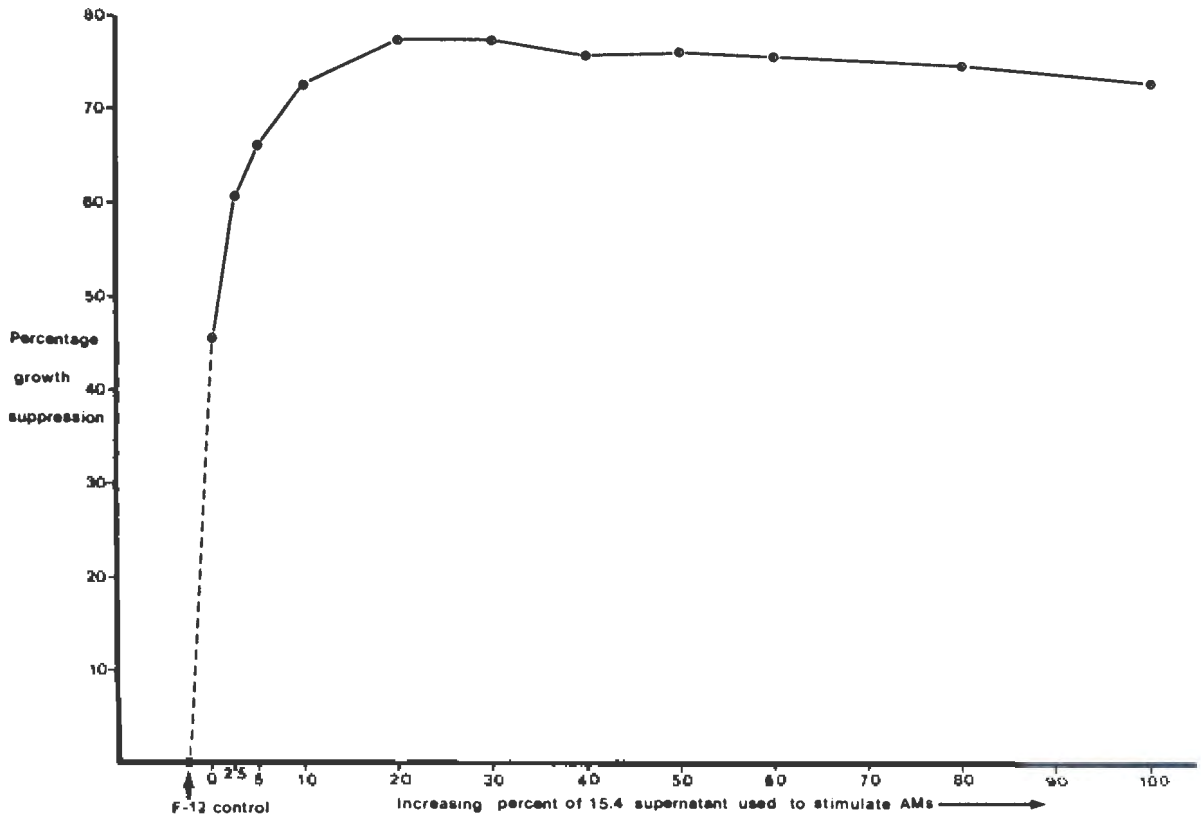


FIG. 1 Effect of increasing the concentration of 15.4 cell supernatant on AMs and subsequent effect on 21.3 cells expressed as percentage growth suppression relative to an F-12 control

obtained from a metrizamide gradient described by Myer *et al.* (1987), except that, in this instance, the diffuse band containing normal type II cells had been removed. The 21.3 cells and fibroblasts were initially seeded in tissue culture wells at 4×10^3 cells/well/0.5 ml culture medium. The fibroblasts were cultured in Modified Eagle's Medium (MEM) containing the same additives as the F-12 nutrient medium used on 21.3 cells.

The tissue culture plates were placed in a 5% CO₂ incubator for 24 h at 37 °C before a twofold dilution series of a 1 × concentrated GIF peak was added to wells containing either fibroblasts or 21.3 cells. The first series of 4 replicates for each of the fibroblasts or 21.3 cell preparations contained a 1:2 dilution of GIF, after which 0.5 ml was transferred serially among the remaining series of 4 replicates for each dilution of 1:4, 1:8 up to 1:128. After a further 24 h, both the fibroblasts and 21.3 cells were assayed for DNA synthesis potential as described previously.

Effect of growth inhibitory/stimulatory factors on normal and transformed CFTH cells

In order to investigate whether or not the growth stimulatory/inhibitory factors had comparable effects on normal (N) and transformed (T) CFTH cells, a twofold dilution series of either a GF or GIF peak was added to each of the respective cultures initially seeded at approximately 4×10^3 cells/tissue culture well/0.5 ml F-12 nutrient medium. Each dilution series began at 1:4 and proceeded up to 1:256 as previously described.

After 24 h, both CFTH(N) and CFTH(T) cells were assayed for DNA synthesis potential as previously described.

RESULTS

Stimulation of normal AMs with a series of dilutions of chemotactic factor

The inhibition of ³H-thymidine incorporation by the

21.3 cells in the presence of supernatant from normal AMs stimulated with different concentrations of 15.4 cell supernatant, relative to that of control cells exposed only to F-12 nutrient medium (F-12 control), is shown in Fig. 1. The position of this control on the x-axis has been chosen arbitrarily, since it was not part of the concentration series and only served to indicate a baseline of 21.3 cell growth in the absence of any supernatant that had been conditioned by unstimulated AMs.

As can be seen, unstimulated AMs (zero concentration on x-axis) produce a GIF that gives a 45.7% growth suppression relative to the F-12 control. However, this was enhanced when the macrophages were stimulated by the chemotactic factor present in the 15.4 cell supernatant. The maximum suppression of 21.3 cell growth occurred when the AMs were stimulated with 30% 15.4 cell supernatant.

Stimulation of whole lung lavage cell population with chemotactic factor

The effect of increasing the concentration of crude supernatant from a chemotactic factor-stimulated AM + Lfc culture (containing both GF and GIF) on 21.3 cells is shown in Fig. 2, where the results are expressed as percentage growth stimulation relative to the F-12 control. As can be seen, there was an essentially linear relationship of growth stimulation with increasing concentration of supernatant up to 1:4, after which there was a decrease in growth stimulation at the 1:2 dilution.

GF and GIF activity of column fractions using tumour target cell assay

The growth suppressive activity of chemotactic factor-stimulated, AM-conditioned medium on 21.3 cells, following fractionation by gel-filtration, is shown in Fig. 3. The results are expressed in terms of percentage growth suppression relative to fraction 32 that was chosen arbitrarily to represent 0% growth suppression. Maximum activity was found in fractions 57–60, corresponding to

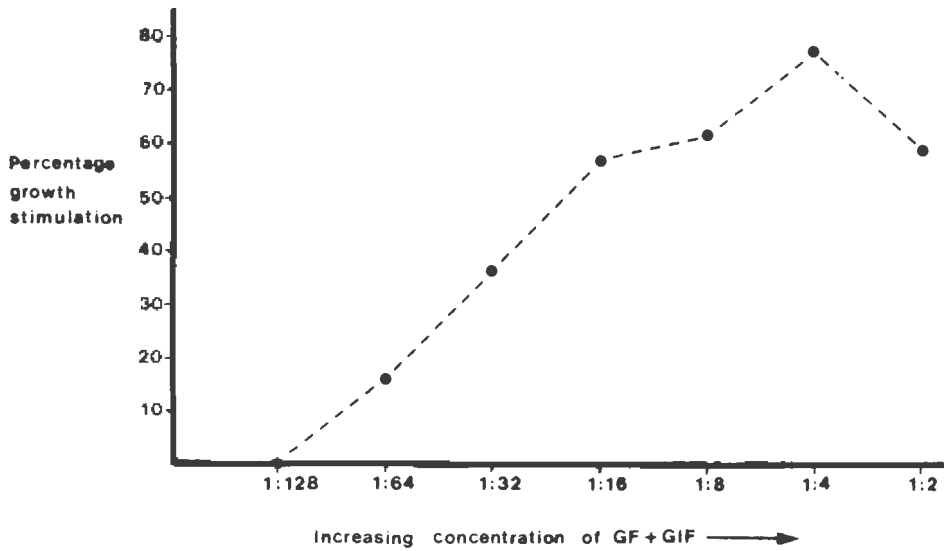


FIG. 2 Effect of increasing the concentration of crude supernatant from a chemotactic factor-stimulated AM + Lfc culture on 21.3 cells expressed as percentage growth stimulation relative to the F-12 control

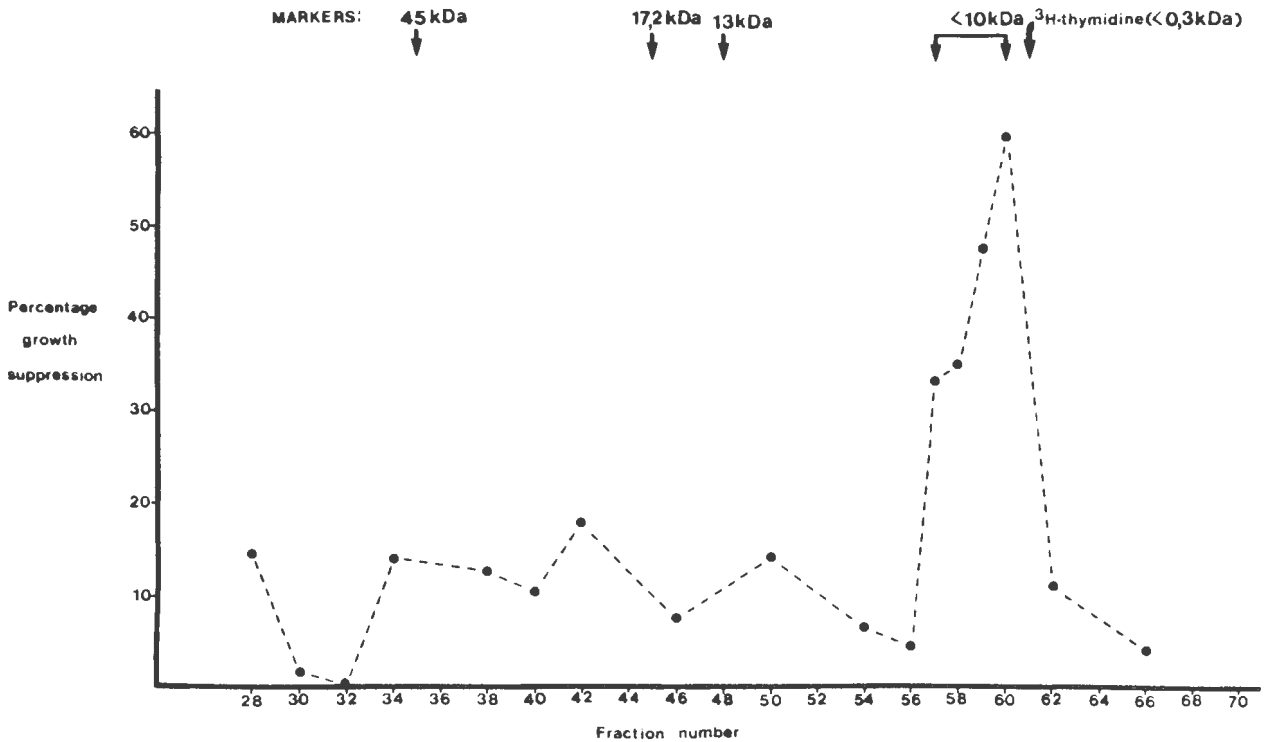


FIG. 3 Effect of fractionated supernatant from chemotactic factor-stimulated AMs on 21.3 cells expressed as percentage growth suppression relative to Sample 32

the position of markers with a molecular mass between 0,3 and 10 kDa.

The growth suppressive/stimulatory activities of the AM-conditioned medium (with and without chemotactic factor stimulation) and the AM + Lfc-conditioned medium (with and without chemotactic factor stimulation) on 21.3 cells following column fractionation, is shown in Fig. 4. The results are expressed as percentage growth suppression/stimulation relative to the F-12 baseline control. Supernatant from AMs cultured without the presence of chemotactic factor (cf) contained a peak of growth stimulatory activity corresponding to a molecular mass in the region of 25,5 kDa. This peak was considerably reduced when AMs were stimulated with chemotactic factor (AM + cf), and a peak of growth suppressive

activity was apparent between the marker positions less than 10 kDa but greater than 0,3 kDa.

The curve of AM + Lfc (without cf-stimulation) shows a similar peak of growth stimulatory activity in the region of 25 kDa as obtained for unstimulated AMs alone, except that a 2nd peak of growth stimulatory activity also occurred at the position of the 13 kDa marker. The curve of AM + Lfc (with cf-stimulation) showed a peak of growth stimulatory activity at the position of the 19 kDa marker and a broad peak of growth suppressive activity between the 0,3 kDa and 10 kDa markers.

The position of the different activity peaks in relation to molecular mass was obtained from a standard curve of log molecular mass versus fraction number for markers of 45 kDa, 17,2 kDa and 13 kDa (curve not shown).

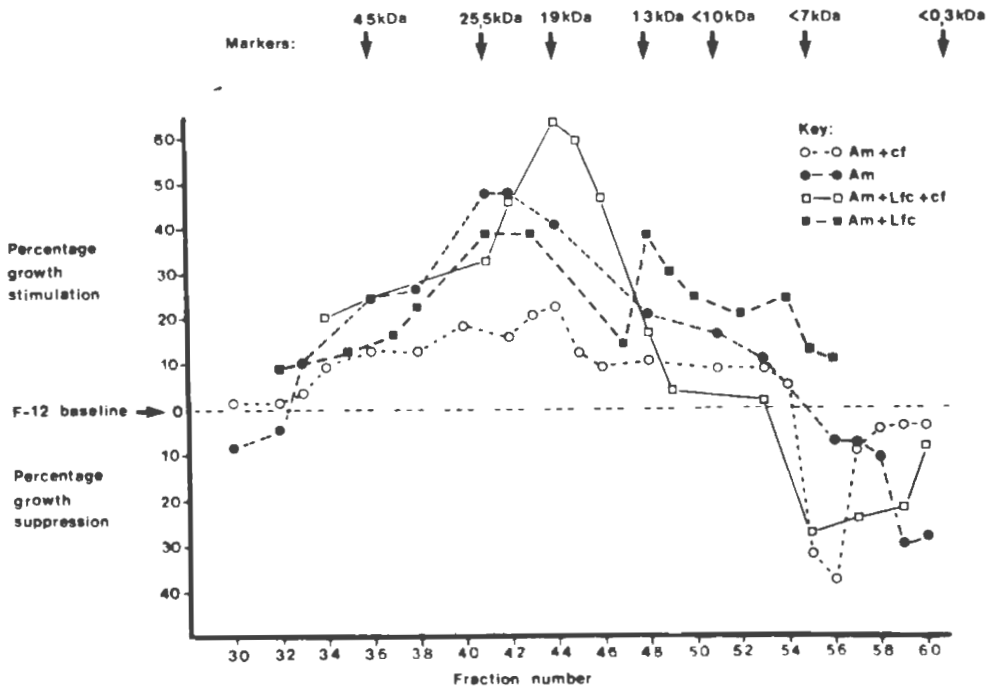


FIG. 4 Effect of fractionated AM supernatant on 21.3 cells expressed as percentage growth stimulation or suppression relative to F-12 control

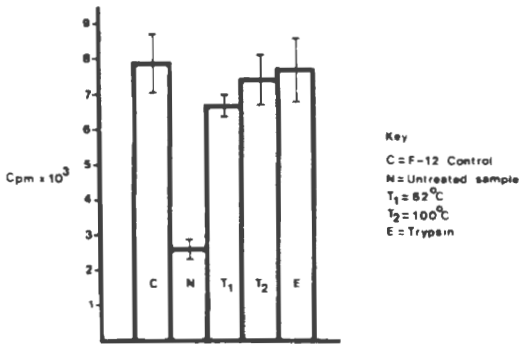


FIG. 5a Effect of trypsin and heat treatment of GIF as measured by a ³H-thymidine incorporation assay using 21.3 cells

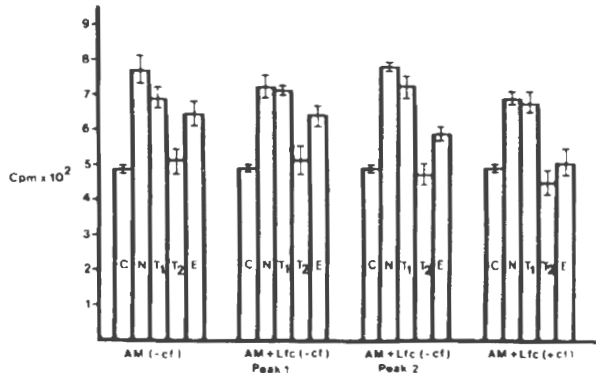


FIG. 5b Effect of trypsin and heat treatment of GF as measured by a ³H-thymidine incorporation assay using 21.3 cells

Sensitivity of growth inhibitory/stimulatory factors to heating and trypsin digestion

The results are depicted in the form of histograms in Fig. 5a & b, where the former is concerned with the treatment of samples of the GIF peak shown in Fig. 3 (pooled fractions 57-60) and the latter with GF peaks in Fig. 4. In Fig. 5a, the amount of ³H-thymidine incorporation by the 21.3 cells is expressed as average counts per

minute (cpm) × 10³. A depression of isotope incorporation reflects GIF activity, as is apparent when comparing the cpm of 21.3 cells in the presence of the untreated sample (N) to the F-12 nutrient medium control (C) where GIF was not present. Samples from the GIF peak were found to be susceptible to heating to 62 °C and 100 °C (T₁ & T₂) as well as to exposure to trypsin (E). In all cases, inactivation of GIF gave cpm values comparable to that of C.

In Fig. 5b, the amount of ³H-thymidine incorporation by the 21.3 cells is expressed as average counts per minute (cpm) × 10². An increase of isotope incorporation reflects GF activity, as is apparent when comparing the cpm of N relative to C, a common control for the 4 series of histograms depicting various treatments of samples from the 4 different GF peaks. In the first histogram, the 25.5 kDa peak (from fractionation of supernatant collected from cultured AMs, no chemotactic factor being present) is shown to be stable at T₁ but labile at T₂. Another sample from this GF peak was marginally sensitive to trypsin relative to N, but was not completely inactivated, as it gave a higher cpm than C.

The next histogram deals with the first peak of fractionated supernatant (also in the region of 25.5 kDa), collected from AM + Lfc cultured without the presence of chemotactic factor (-cf). The GF was again found to be stable at 62 °C, labile at 100 °C and marginally sensitive to trypsin. The 3rd histogram demonstrates that the 2nd peak of fractionated supernatant (in the region of 13 kDa, Fig. 4) differs from the previous one only in being more sensitive to trypsin.

The 4th histogram deals with samples from the GF peak in the region of 19 kDa (Fig. 4), from fractionated supernatant collected from AM + Lfc cultured in the presence of chemotactic factor (+cf). This peak was found to be equally sensitive to T₂ and to exposure to trypsin, having cpm values comparable to that of C.

DNA synthesis potential of fibroblasts and 21.3 cells treated with a dilution series of growth inhibitory/stimulatory factors

The effect of increasing the concentration of GIF on sheep lung fibroblasts and 21.3 cells is shown in Fig. 6.

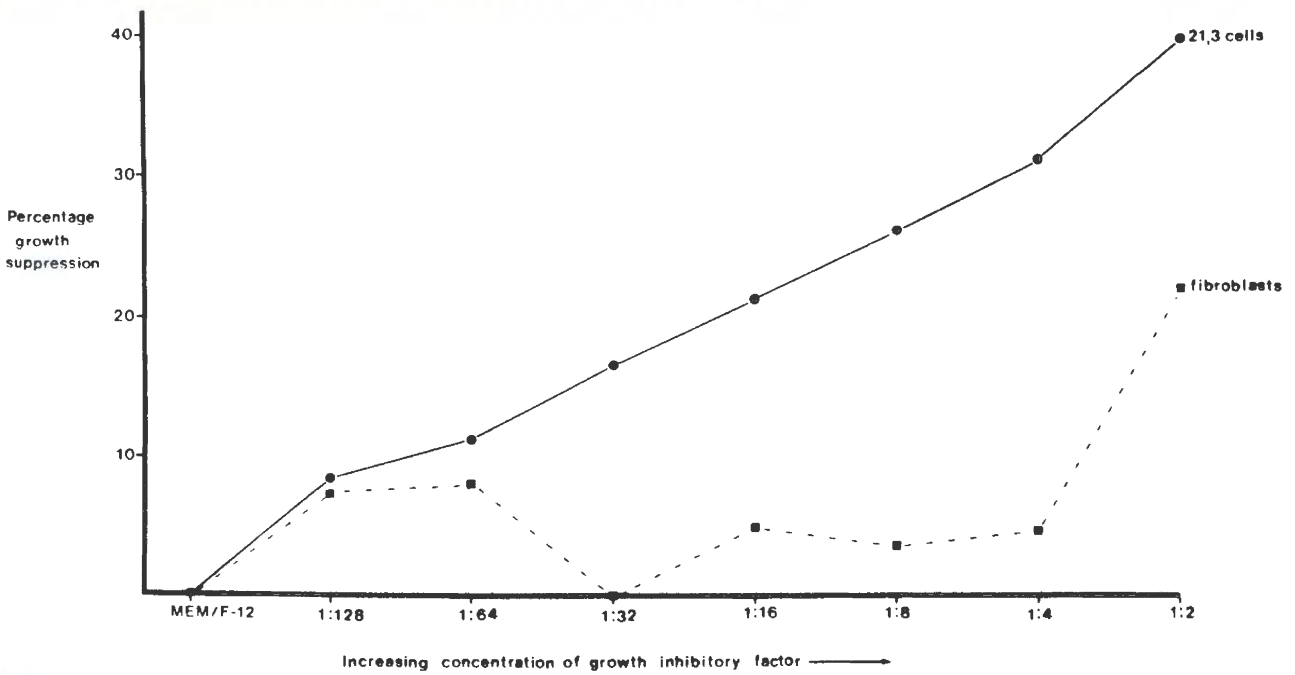


FIG. 6 Effect of increasing the concentration of GIF on fibroblasts and 21.3 cells expressed as percentage growth suppression relative to MEM or F-12 control

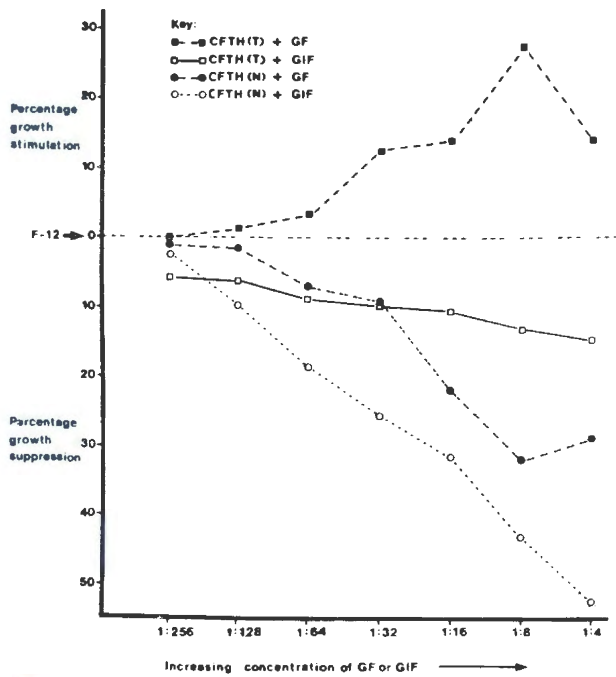


FIG. 7 Effect of increasing concentrations of GF and GIF on normal (N) and transformed (T) calf foetal thyroid (CFTH) cells expressed as percentage growth stimulation or suppression relative to the F-12 control

The results are expressed as percentage growth suppression relative to the control cells exposed only to Modified Eagles Medium (MEM) or F-12 control, respectively, for each series dilution. As can be seen, there is an essentially linear relationship between the growth suppression in 21.3 cells with increasing concentration of GIF, while the fibroblasts were relatively unaffected by the GIF except at the 1:2 dilution.

Effect of growth inhibitory/stimulatory factors on normal and transformed CFTH cells

The effect of increasing concentrations of AM-derived GF (25,5 kDa peak from supernatant from unstimulated AMs, Fig. 4) and GIF (peak between 0,3 kDa and 10

kDa, from supernatant from cf-stimulated AMs, Fig. 4) on normal and transformed CFTH cells, is shown in Fig. 7, where the results are expressed as percentage growth suppression or stimulation relative to the F-12 control. As can be seen, CFTH (N) cells were more susceptible than the CFTH (T) cells to the effects of increasing the concentration of GIF. CFTH (N) responded in a negative fashion to increasing concentration of GF compared to CFTH (T) which were maximally responsive to GF (in terms of percentage growth stimulation) at a dilution of 1:8 with a decreased response at 1:4 concentration.

DISCUSSION

Macrophages produce a variety of growth stimulatory and inhibitory factors, and hence the possibility of AMs producing one or more of these substances affecting jaagsiekte tumour cell growth was assessed.

Increasing concentrations of crude supernatant collected from chemotactic factor-stimulated AMs was found to have a progressively inhibitory effect on jaagsiekte tumour cell growth (Fig. 1), although unstimulated AMs were also shown to produce this factor, but to a lesser extent. Thus, the effect of the chemotactic factor in this instance was to enhance the production of GIF by normal AMs. Fractionation of this GIF, using a Sephadex G-75 column, revealed a peak of activity in the region between 0,3 kDa and 10 kDa (Fig. 3).

Normal AMs cultured without the presence of chemotactic factor also produced a tumour cell growth factor with a molecular mass in the region of 25,5 kDa (Fig. 4). When Lfcs were added to this culture system, a second peak of GF activity occurred at the 13 kDa marker position in addition to the 25,5 kDa peak (Fig. 4). Although both GF (19 kDa, Fig. 4) and GIF (between 0,3 kDa and 10 kDa, Fig. 4) was present in the fractionated supernatant collected from chemotactic factor-stimulated AM + Lfc cultures, the overall effect of the crude supernatant was to stimulate jaagsiekte tumour cell growth (Fig. 2). The GIF peak in Fig. 4 is comparable to the peak obtained in Fig. 3, except that in the latter case no percentage growth stimulation was apparent for any of the other column samples, since percentage growth suppression was expressed relative to fraction 32, and not relative to

an F-12 control. Thus, the 60 % peak fraction in Fig. 3 would probably have also been closer to 38 % as obtained in Fig. 4, had an F-12 control been included.

Szaniawska *et al.* (1985) found both tumour cell growth stimulatory and inhibitory factors in their conditioned medium collected from whole rat lung culture material. Fractionation of this supernatant followed by a ³H-thymidine incorporation assay using tumour cells, revealed the GFs to be in the 30–70 kDa region and the GIFs in the 12–20 kDa and 3–5 kDa regions. They suspected that the last-mentioned GIF could have been smaller than 3 kDa, as the ability of the Sephadex G-75 column to separate low molecular mass species is rather limited. In our study, the GIF peak on a Sephadex G-75 column lay between the 0.3 kDa and 10 kDa marker positions.

The fact that Szaniawska *et al.* (1985) also found GFs in their fractionated conditioned medium probably resulted from the presence of other cell types interacting with the AMs, such as suggested by Kovaks & Kelly (1985), where sensitized T-helper cells secrete a lymphokine which can activate AMs to release a macrophage-derived growth factor (MDGF). Skimokado *et al.* (1985) have indicated that a significant part of the MDGF consists of at least 2 forms of platelet-derived growth factor (PDGF), namely, a size class of approximately 37–39 kDa and another between 12 and 17 kDa. In the present study, normal AMs with and without the presence of Lfc (containing mainly lymphocytes) were found to produce a GF in the region of 25.5 kDa, although this GF activity was lost following chemotactic factor stimulation of the AMs unless the Lfc fraction was present (Fig. 4). In the latter case, the GF peak was in the region of 19 kDa, and not in the 25.5 kDa region. It is thus possible that the 13 and 19 kDa peaks are a result of interaction between T-lymphocytes and AMs, since these peaks are close to the size range of the smaller form of PDGF between 12 and 17 kDa.

Both peaks in the 25.5 kDa region (from unstimulated AMs or unstimulated AM + Lfc cultures) showed the same response to trypsin and heat treatment, being labile only to heating to 100 °C (Fig. 5b), whereas the 19 kDa peak sample was also susceptible to trypsin, as was the 2nd GF peak obtained from unstimulated AM + Lfc cultures (in the 13 kDa region, Fig. 4). Thus, on the basis of heating and enzyme treatment, the GFs are polypeptide in nature, and the 2 GF peaks in the 25.5 kDa region appear to differ from those in the 13 and 19 kDa regions, implying that the former are AM-derived, and the latter from either the Lfc itself or in conjunction with the AMs.

Bottazzi *et al.* (1985) have speculated on the role of tumour-derived chemotactic factors in promoting tumour growth. Meltzer, Stevenson & Leonard (1977) were able to show that tumour-derived chemotactic activity correlated well with phases of accelerated growth of 5 tumour cell lines grown *in vitro*, and suggested that chemotactic factors released by tumours could be a means whereby they regulate their local environment with respect to macrophage accumulation. In the present study, chemotactic factor-stimulation of normal AMs appeared to result in the production of a GIF, whereas when the Lfc fraction was present, the GF overrode the effect of GIF (Fig. 2). Thus, in an attempt to apply these *in vitro* results to the *in vivo* situation in jaagsiekte sheep lungs, it is suggested that chemotactic factor-stimulation of normal AMs results in the overall production of a tumour cell growth factor mediated by components of the Lfc fraction.

The GIF, as characterized in this study, did not have any growth stimulatory effect on sheep lung fibroblasts (Fig. 6), which was in contrast to the findings of Vilcék, Palombella, Henriksen-DeStefano, Swenson, Feinman, Hirai & Tsujimoto (1986) who found that a highly purified form of *E. coli*-derived recombinant human tumour necrosis factor stimulated the growth of lung and skin fibroblasts in a manner similar to that of the action of interleukin 1, which is believed to be a potential regulator of fibroblast proliferation (Schmidt, Mizel, Cohen & Green, 1982).

Tumour necrosis factor (TNF) is a monocyte/macrophage-derived protein that is cytotoxic or cytostatic for some tumour cell lines and stimulates the growth of non-transformed cells and fibroblasts (Vilcék *et al.*, 1986). The GIF isolated from the supernatant of chemotactic factor-stimulated AMs (present study) failed to enhance sheep lung fibroblast growth (Fig. 6) and did not stimulate the growth of non-transformed CFTH cells (Fig. 7). Although this GIF did depress the growth of transformed CFTH cells (to a much lesser extent than that of normal CFTH cells), it probably does not belong to the same type of leukokine peptide as the TNF described by Vilcék *et al.* (1986).

The AM-derived growth factor, however, did stimulate the growth of transformed CFTH cells compared to their non-transformed counterparts (Fig. 7). This dual role of GF in stimulating the growth of transformed CFTH cells but suppressing the growth of normal CFTH cells, could possibly be explained in parallel to the mechanism of action of TNF as postulated by Hatakeyama, Minamoto, Uchizama, Hardy, Yamada & Taniguchi (1985). According to these workers TNF acts on fibroblasts as if it were a growth factor, whereas the same growth factor-receptor interactions on the surface of neoplastic cells could lead to reverse signal transduction, producing growth inhibition instead of growth stimulation, as seen in normal cells. A similar situation could exist in the case of GF in the CFTH system, except that the inhibitory effects of GF on normal CFTH cells is reversed on transformed CFTH cells, resulting in growth stimulation of the latter.

Vilcék *et al.* (1986) have also suggested that the binding of TNF to cell surface receptors might trigger a similar series of biochemical events as seen with other growth factors, such as epidermal growth factor, PDGF and transforming growth factor. Such events are thought to involve the action of protein kinase (Cohen, Carpenter & King, 1980) and induction of cellular genes involved in the regulation of the cell cycle, including *c-myc* (Armelin, Armelin, Kelly, Stewart, Leder, Cochran & Stiles, 1984) and *c-fos* (Greenberg & Ziff, 1984).

The whole picture of the interaction between AMs and transformed type II cells in jaagsiekte sheep lungs cannot be completed at this stage, however, for the following reasons:

- (1) The CFTH culture system was chosen in place of a control consisting of normal type II cells owing to difficulties in obtaining long term cultures of the latter.
- (2) The action of GF and GIF on normal CFTH cells may not be paralleled by the response of normal type II cells to these 2 factors.
- (3) The precise derivation of GFs when Lfcs are present in the AM culture system may not only be attributed to the interaction between AMs and lymphocytes.

Nevertheless, similar responses of neoplastic type II and CFTH cells to the GF presents an overall picture of a tumour-cell-derived-macrophage-chemotactic factor stimulating the production of a tumour-cell-growth factor via an interaction between AMs and other cells present in

the Lfc fraction. Additional work will have to be done in separating the components of the Lfc fraction before the precise origin of the tumour cell-growth factor, following chemotactic factor release by the tumour cells, can be determined for the *in vivo* situation.

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