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Modulation of intercellular communication between smooth muscle cells by growth factors and cytokines

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Abstract

We recently reported that tumor necrosis factor α is able to cause a dose-dependent and persistent reduction in gap junctional intercellular communication between primary human smooth muscle cells. In order to study whether this observed persistent reduction in gap junctional intercellular communication is a unique feature for tumor necrosis factor α, the present study focuses on the effects of other growth factors and cytokines on gap junctional intercellular communication. Platelet-derived growth factor AA and BB (PDGF-AA, PDGF-BB), basic fibroblast growth factor (bFGF), interleukin-6 and interferon-γ were able to modulate gap junctional intercellular communication between primary human smooth muscle cells in vitro. However, our results demonstrate that the magnitude and nature of the observed effects are growth factor- and cytokine-specific. PDGF-AA, PDGF-BB and interleukin-6 caused a transient reduction in gap junctional intercellular communication, while bFGF induced a transient increase in gap junctional intercellular communication. Interferon-γ was shown to be capable of causing a persistent reduction in gap junctional intercellular communication. In addition, PDGF-AA, PDGF-BB, bFGF, interleukin-6, interferon-γ and tumor necrosis factor α all stimulated smooth muscle cell proliferation. These observations suggest a more complex relationship between modulation of gap junctional intercellular communication and cell proliferation than current hypotheses imply. The implications of the observed effects of growth factors and cytokines on gap junctional intercellular communication between smooth muscle cells in relation to the process of atherosclerosis is discussed.

Keywords: Atherosclerosis; Cell proliferation; Cytokine; Gap junctional intercellular communication; Growth factor; Smooth muscle cell

1. Introduction

Atherosclerosis is a pathophysiological phenomenon with a slow progression giving rise to myocardial and cerebral infarctions. Atherosclerotic lesions ('plaques') are characterized as focal thickenings of the intimal layer of the artery wall. Lesion progression is accompanied by infiltration of monocytes and lymphocytes, proliferation of smooth muscle cells, accumulation of intra- and extracellular lipids and synthesis of extracellular matrix components.

Regarding the etiology of atherosclerosis, both the response-to-injury theory and the monoclonal hypothesis focus on disturbance of growth control mechanisms as the key event in atherogenesis. Consequently, both theories assume an important modulatory role for growth factors and cytokines in the pathogenesis of the disease. The response-to-injury theory (Ross, 1993) supposes that growth factors and cytokines produced by cell types present in the lesions, act in an autocrine and/or paracrine manner on smooth muscle cell proliferation. The monoclonal hypothesis (Benditt, 1978) parallels atherogenesis with tumorigenesis, thereby dividing the pathogenesis of plaque formation in stages of initiation and promotion.

Hybridization and immunohistochemical studies demonstrated that platelet-derived growth factor AA and BB (PDGF-AA, PDGF-BB; Salomon et al., 1992), basic fibroblast growth factor (bFGF; Hughes et al., 1993), interleukin-6 (Seino et al., 1994), interferon-γ (Hansson et al., 1989b) and tumor necrosis factor α (Barath et al., 1990) are present in human atherosclerotic plaques. It is not completely clear whether the amount of all these growth modulating substances is elevated in plaques when compared with the nonatherosclerotic vessel wall. bFGF,

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interleukin-6, PDGF and tumor necrosis factor α are able to induce smooth muscle cell proliferation (Raines and Ross, 1993). It has been reported that interferon-γ either stimulates (Yokota et al., 1992) or suppresses (Hansson et al., 1989a; Warner et al., 1989) smooth muscle cell proliferation, probably depending on the culture conditions for these cells.

In vitro and in vivo studies provide evidence for the involvement of modulation of gap junctional intercellular communication in the process of tumor promotion (Yamasaki, 1990). Altered gap junctional intercellular communication seems to be important in the pathogenesis of atherosclerosis as well. Atherogens like oxidized low density lipoprotein, cigarette smoke condensate, oxysterols and aldehydes are able to reduce gap junctional intercellular communication between smooth muscle cells (De Haan et al., 1994; Zwijsen et al., 1990, 1991a,b).

Previous work (Mensink et al., 1995) demonstrated that tumor necrosis factor α is able to cause a dose-dependent and persistent reduction in gap junctional intercellular communication between primary human smooth muscle cells, suggesting that tumor necrosis factor α may act as an endogenous 'promoter' on human smooth muscle cells. In order to study whether this observed persistent reduction in gap junctional intercellular communication between smooth muscle cells is a unique feature for tumor necrosis factor α or a more common event in growth factor or cytokine action on these cells, the present study focuses on the effects of PDGF-AA and PDGF-BB, bFGF, interleukin-6 and interferon-γ on gap junctional intercellular communication between primary human smooth muscle cells. Furthermore, we have studied the effect of these growth modulating peptides and tumor necrosis factor α on smooth muscle cell proliferation in our cell culture system, in order to obtain more knowledge about the relationship between gap junctional intercellular communication and cell proliferation.

2. Materials and methods

Recombinant human tumor necrosis factor α (molecular weight 36 kDa; specific activity 10^8 U/mg), recombinant human interleukin-6 (molecular weight ≈ 26 kDa; specific activity 1.8×10^8 and 1.8×10^9 U/mg) and recombinant human interferon-γ (molecular weight 34 kDa; specific activity 2.5×10^7 U/mg) were obtained from Genzyme Diagnostics (Cambridge, MA, USA). PDGF-AA, PDGF-BB and bFGF were obtained from Genzyme Diagnostics (Cambridge, MA, USA; molecular weight PDGF 26 kDa, molecular weight bFGF 17.5 kDa) and from Gibco BRL (Paisley, UK, molecular weight PDGF 30 kDa, molecular weight bFGF 17 kDa). Cell proliferation kits based on 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) cleavage by mitochondrial dehydrogenases and NADH were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Eagle's minimum essential medium (modified) with Earle's salts (EMEM) was purchased from ICN Biomedicals, Costa Mesa, CA, USA. Fetal calf serum, gentamicin and fungizone were obtained from Gibco BRL (Paisley, UK). Dulbecco's 'A' phosphate-buffered saline (PBS) was from Oxoid Ltd. (UK). Trypsin 1 : 250 was from Difco (USA). Sodium pyruvate was from BDH Chemicals (UK). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (Hepes), potassium phosphate, lithium chloride and acetic acid were purchased from Merck (Darmstadt, Germany). Triton X100, Lucifer yellow CH, and bovine serum albumin fraction V were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.1. Culture of primary human smooth muscle cells

Primary human smooth muscle cells were isolated from arteries of human umbilical cords by an explant technique. After careful removal of the adventitial layer, the arterial tissue was cut into small pieces. Explants were incubated in EMEM supplemented with 10% fetal calf serum, 50 μg/ml gentamicin and 1.25 μg/ml fungizone in a 37°C, 5% CO_2 humidified atmosphere. Cells were allowed to grow for 3–4 weeks until subculturing. Cells displayed the well known 'hills and valleys' pattern after reaching confluence (Ross and Kariya, 1983). Experiments were performed on cells in passage 2–5.

2.2. Experimental procedure

For measurement of gap junctional intercellular communication, smooth muscle cells were grown in 35 mm culture dishes (Greiner, Alphen a/d Rijn, Netherlands) until confluency. Serum containing culture medium was removed whereafter cells were washed with PBS. EMEM supplemented with antibiotics and 0.1–0.5% bovine serum albumin was added to the cells. Cells were then incubated with the growth modulating factors or with either PBS (in the case of interleukin-6, interferon-γ, bFGF) or acetic acid (in the case of PDGF) as appropriate blanks. Just prior to gap junctional intercellular communication measurement, Hepes buffer (pH 7.4) was added to the incubations (final concentration 20 mM) to stabilize the pH during microinjection and fluorescence microscopy.

2.3. Measurement of gap junctional intercellular communication

Gap junctional intercellular communication was determined after microinjection of a 20% Lucifer Yellow CH (in 0.33 M lithium chloride) solution in a single cell (Enomoto et al., 1984). In each smooth muscle cell culture at least 20 individual cells were microinjected using a vertical injection system (Olympus Injectoscope IMT-2-syf) (Yamamoto and Furusawa, 1978) with a dye filled capillary glass tip (Clark, Pangbourne, UK). The capillary

glass tip was prepared using an automatic magnetic puller (Narishige, Tokyo, Japan) with a tip diameter of 1 μm. The Lucifer Yellow CH filled cells were checked with phase-contrast and fluorescence microscopy directly after microinjection. 15–20 min after the first injection the number of communicating cells was determined using fluorescence microscopy. The average number of fluorescent cells was calculated for each incubation. The average number of communicating cells in control incubations was taken as 100% gap junctional intercellular communication. Each experiment was performed in duplicate. At least three independent tests were done. Data presented in Figs. 1–4 are the average values of these tests.

2.4. Cell proliferation assays

5000–10000 smooth muscle cells ($n = 8$) were plated in serum containing culture medium in 96 wells microtiter plates (tissue culture grade, Greiner, Alphen a/d Rijn, Netherlands); 100 μl medium per well. After 24 h, the culture medium was removed and the cells were washed with PBS. EMEM supplemented with antibiotics and 0.5% bovine serum albumin was added to the cells. Cells were then incubated with the growth modulating factors or with either PBS or acetic acid as appropriate blanks for 24 h, whereafter cell proliferation was measured. After incubation with growth modulating factors, smooth muscle cells were incubated with MTT reagent according to the instructions of the manufacturer. The MTT colorimetric assay is based on cleavage of the yellow tetrazolium salt MTT to formazan dye by dehydrogenase activity in active mitochondria of living cells. The formazan dye formed is quantified using a Thermomax microplate reader (Molecular Devices, Sunnyvale, CA, USA). An increase in number of living cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample, which correlates to the amount of formazan formed as monitored by the absorbance at 595 nm.

2.5. Cytotoxicity assay

Smooth muscle cells were grown in six-well tissue culture plates (Costar Europe). When confluent, cells were incubated as for gap junctional intercellular communication measurement. After treatment, media were taken from the wells and were centrifuged. Cells were scraped from the bottom of the wells after addition of 0.5% Triton X-100 in 0.1 M phosphate buffer, pH 7.5, followed by a sonification step in ice water (5 min, Sonorex RK 100 (Bandelin GmbH, Berlin, Germany)) and a centrifugation step. Lactate dehydrogenase (LDH) activity was measured in the supernatants (Mitchell et al., 1980) and LDH leakage was calculated. Each experiment was performed at least in duplicate and at least two independent tests were done.

2.6. Statistics

Statistical analyses of the data were performed using Student's t-test ($P < 0.05$).

3. Results

3.1. Gap junctional intercellular communication between smooth muscle cells

Primary human smooth muscle cells display a distinct gap junctional intercellular communication. In typical experiments Lucifer yellow spread over an average of 15–80 cells (probably depending on cell density at confluency) in control incubations. In most experiments a control value of ~ 40 communicating cells was observed.

Within separate experiments, standard deviations for duplicate incubations were generally small. The standard deviations for control incubations and growth factor or cytokine incubations were quite similar within experiments. In experiments with interferon-γ, bFGF and interleukin-6 the average standard deviations within single experiments were 3–5%. In experiments with PDGF results were more variable; standard deviations were a few percent higher than in the other experiments. Standard deviation values in the order of 10% were seen in a number of cases in separate PDGF experiments.

3.1.1. PDGF

PDGF-AA and PDGF-BB reduce gap junctional intercellular communication between primary human smooth muscle cells in a similar way. In 1 h incubations, gap junctional intercellular communication is reduced to ~ 80% of control value at 0.1 nM PDGF (Fig. 1A,B). Higher concentations PDGF-AA and PDGF-BB resulted in a stronger decrease in gap junctional intercellular communication, with a maximal inhibition of 35–40%, reached at 0.5–0.7 nM PDGF. The observed inhibition in gap junctional intercellular communication is transient for both PDGF isoforms with a maximum inhibition after 1 h incubation followed by a return to almost control values within 24 h (Fig. 2A,B). Replacement of PDGF containing culture medium with medium containing fresh PDGF after 3 h during an exposure time of 4 h did not give rise to different experimental outcomes for both PDGF-AA and PDGF-BB isoforms (data not shown). Thus, the transient nature of this gap junctional intercellular communication inhibition upon incubation with PDGF isoforms appeared not to be caused by a depletion of PDGF in the culture medium.

3.1.2. Interleukin-6

One hour incubations of confluent smooth muscle cell cultures with 0.5 nM interleukin-6 did not result in a consistent reduction in gap junctional intercellular commu-

nication between these cells (Fig. 3A). Incubation with 2.5 and 5.0 nM interleukin-6 for 1 h caused a small but significant reduction in gap junctional intercellular communication of 13 ± 4.5 and 17 ± 6.0%, respectively. However, when smooth muscle cells were incubated for a longer period of time with 0.5 nM interleukin-6, a significant reduction in gap junctional intercellular communication was obtained at 7 and 9 h of exposure (Fig. 3A). At these time points, gap junctional intercellular communication was inhibited to ~ 80% of control incubations. At 17 and 24 h of incubation with 0.5 nM interleukin-6, gap junctional intercellular communication was restored to control values (Fig. 3A). Again, the transient nature of this gap junctional intercellular communication inhibition by interleukin-6 appeared not to be a consequence of a depletion of the cytokine in the culture medium, since a 17 h incubation time resulted in the same gap junctional intercellular communication value as a 10 + 7 h incubation with refreshment of interleukin-6 containing culture medium after 10 h of incubation (data not shown).

3.1.3. Interferon-γ

Upon addition of 0.5 nM interferon-γ to smooth muscle cells, gap junctional intercellular communication is reduced to ~ 80% of control incubations (Fig. 3B). This reduction in gap junctional intercellular communication occurred within 1 h and lasted for at least 24 h. When 2.0 or 3.5 nM interferon-γ were added to the smooth muscle cells for 1 h, gap junctional intercellular communication was not further reduced, i.e. 20 ± 0.7 and 18 ± 1.4% inhibition respectively.

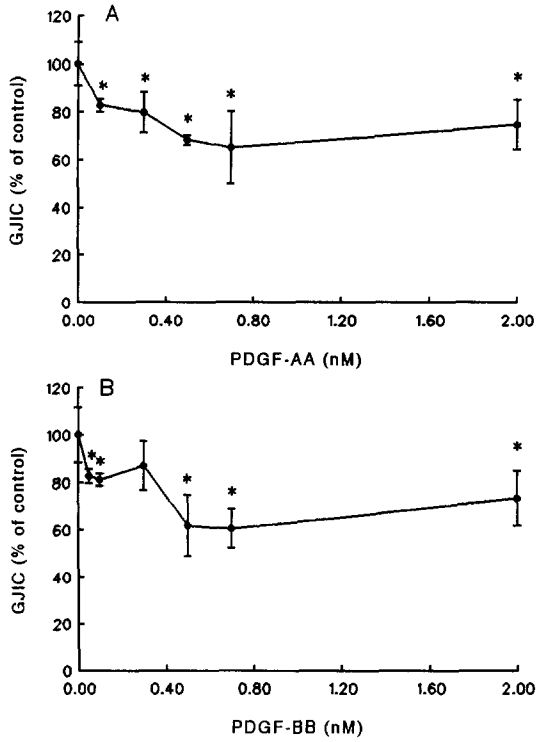

Fig. 1. Dose-dependent inhibition of gap junctional intercellular communication between human smooth muscle cells by PDGF-AA (A) or PDGF-BB (B). Exposure time was 1 h to the indicated concentrations of PDGF-AA or PDGF-BB. All values are means ± S.D. * Significantly different from control value.

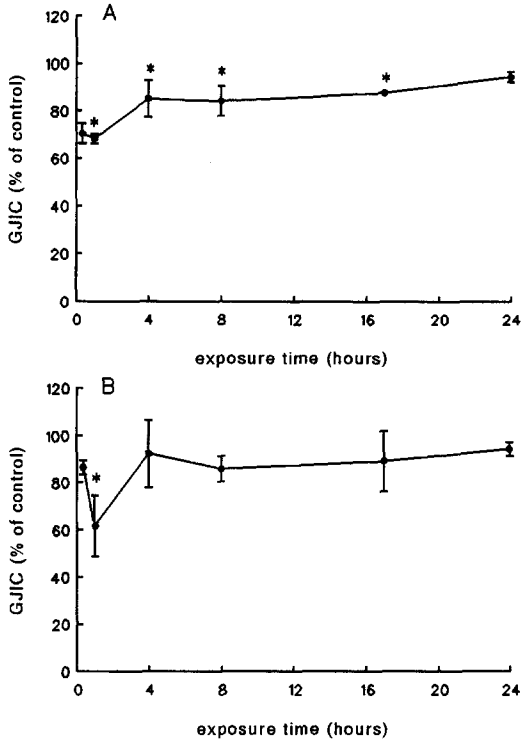

Fig. 2. Time-dependent inhibition of gap junctional intercellular communication between human smooth muscle cells by PDGF-AA (A) or PDGF-BB (B). Exposure concentration was 0.5 nM PDGF-AA or 0.5 nM PDGF-BB for the indicated periods of time. All values are means ± S.D. Average S.D. for control incubations was 9.0% (A) and 11.7% (B). * Significantly different from control value.

3.1.4. bFGF

Addition of 0.5 nM bFGF to smooth muscle cells resulted in a significant increase in gap junctional intercellular communication to 123% of control incubations at 24 h of exposure only (Fig. 4A). At 30 h incubation, gap junctional intercellular communication returned to control value, irrespective of addition of fresh bFGF containing culture medium after 6 h incubation (data not shown). Incubation of smooth muscle cells with 2.0 and 4.0 nM bFGF for 24 h resulted in a further increase in gap junctional intercellular communication; with a doubling of the number of communicating smooth muscle cells at 4.0 nM (Fig. 4B).

3.2. Cytotoxicity

To determine whether the observed effect on smooth muscle cell gap junctional intercellular communication was influenced by loss of membrane integrity upon incubation with growth factors or cytokines, LDH activity was measured in culture media and cell homogenates. No

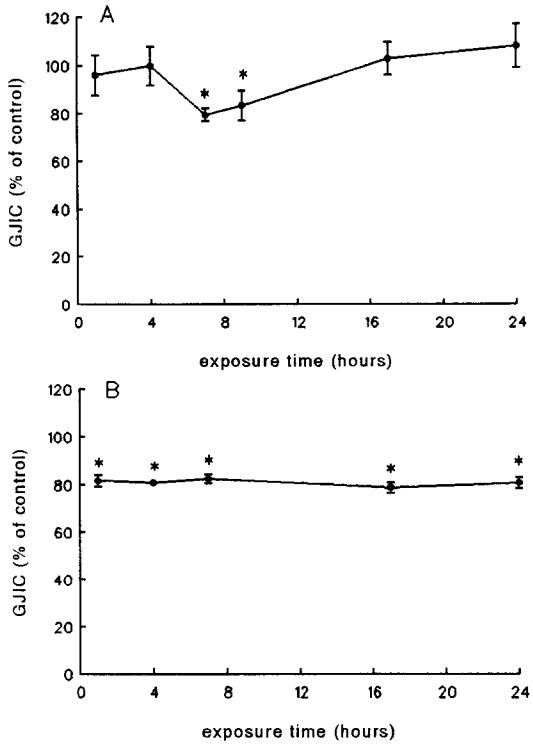

Fig. 3. Gap junctional intercellular communication between human smooth muscle cells upon incubation with 0.5 nM interleukin-6 (A), or 0.5 nM interferon-γ (B) for the indicated periods of time. All values are means ± S.D. Average S.D. for control incubations was 3.2% (A) and 2.8% (B). * Significantly different from control value.

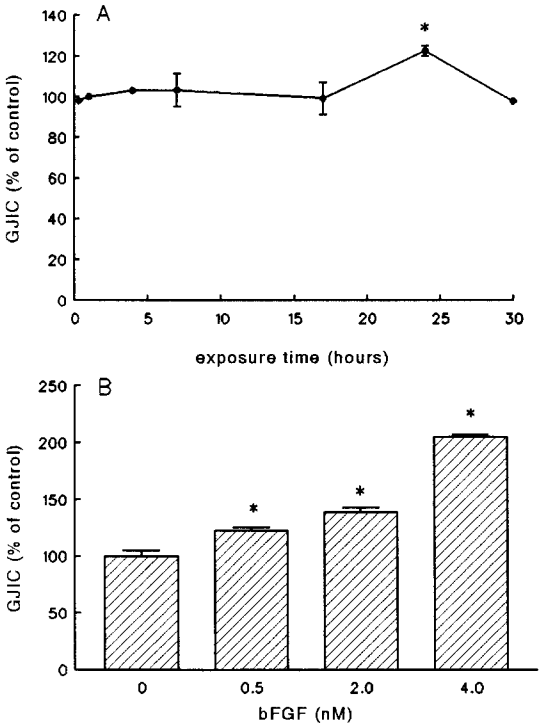

Fig. 4. Gap junctional intercellular communication between human smooth muscle cells upon incubation with 0.5 nM bFGF for the indicated periods of time (A) or after exposure for 24 h to the indicated concentrations of bFGF (B). All values are means ± S.D. Average S.D. for control incubations was 4.8% (A). * Significantly different from control value.

Table 1
Maximal absorbance values of smooth muscle cell incubations with growth factors and cytokines as measured in the MTT test after incubation with these factors for 24 h

Growth factor/cytokine	Absorbance 595 nm (percentage of control)
PDGF-AA	118 ± 13 [a]
PDGF-BB	132 ± 17 [a]
Interleukin-6	109 ± 9 [a]
bFGF	107 ± 9 [a]
Tumor necrosis factor α	140 ± 20 [a]
Interferon-γ	144 ± 19 [a]

Values are means ± S.D. ($n = 8$). S.D. for control incubations varied from 2 to 8%. [a] Significantly different from control value.

membrane cytotoxicity was observed in smooth muscle cell cultures treated for 24 h with 0.5 or 2.5 nM interleukin-6, 0.5 or 0.7 nM PDGF-AA or PDGF-BB, or 0.5 nM interferon-γ (data not shown). Treatment of cells for 30 h with 0.5 nM bFGF did not cause membrane cytotoxicity either. Thus, these studies on gap junctional intercellular communication were done at noncytotoxic concentrations.

3.3. Smooth muscle cell proliferation

In a number of experiments, PDGF-AA, PDGF-BB, interleukin-6, bFGF, tumor necrosis factor α and interferon-γ were able to stimulate smooth muscle cell proliferation. In Table 1 maximal absorbance values (percentages of control) are given for the incubations of smooth muscle cells with growth factors and cytokines. However, a considerable inter-experimental variance was observed (data not shown). In some 24 h incubations with growth factors and cytokines, no significant cell proliferation was observed compared to control incubations. In some other experiments, PDGF-AA and PDGF-BB could not induce cell proliferation, whereas the other growth modulating factors did stimulate smooth muscle cell growth. Furthermore, the magnitude of the proliferative response varied strongly per experiment. In some experiments tumor necrosis factor α and interferon-γ were the most potent factors tested; in other experiments the tested growth factors and cytokines seemed to be more or less equally potent. Experiments in which smooth muscle cells were incubated for 72 h with the growth modulating factors also resulted in the above discussed forms of inter-experimental variance (data not shown). In our cell culture system, we never observed a suppression of smooth muscle cell growth upon incubation with interferon-γ.

4. Discussion

The present study demonstrates that the growth modulating factors PDGF-AA, PDGF-BB, interleukin-6, bFGF and interferon-γ are able to modulate gap junctional inter-

cellular communication between primary human smooth muscle cells. In the case of PDGF-AA, PDGF-BB and interleukin-6, a transient reduction in gap junctional intercellular communication is observed. On the contrary, a transient increase in gap junctional intercellular communication is seen in smooth muscle cells incubated with bFGF. Furthermore, interferon-γ is shown to be a growth modulating factor capable of causing a persistent reduction in gap junctional intercellular communication (> 24 h).

Time course effects of interferon-γ show a resemblance with the effects of tumor necrosis factor α on gap junctional intercellular communication between human smooth muscle cells (Mensink et al., 1995). The ~ 20% inhibition in gap junctional intercellular communication caused by interferon-γ is, however, lower than the ~ 40% inhibition caused by tumor necrosis factor α, but both factors cause a persistent reduction in gap junctional intercellular communication for at least 24 h.

Maldonado et al. (1988) reported that PDGF (isoform not mentioned) reduced gap junctional intercellular communication at 20 min after growth factor application with 0–37%, depending on the cell type used. In a dose-response curve at 20 min after addition of PDGF, they observed a 37% inhibition of gap junctional intercellular communication between BalbC 3T3 cells upon incubation with 0.6 nM PDGF. This corresponds well with the 35–40% inhibition in gap junctional intercellular communication between smooth muscle cells seen in our experiments upon incubation with 0.5–0.7 nM PDGF. Pelletier and Boynton (1994) demonstrated however, that 0.3 nM PDGF-BB inhibits gap junctional intercellular communication between C3H/10T1/2 cells almost completely after 40 min incubation. The transient nature of gap junctional intercellular communication inhibition observed in our experiments upon PDGF exposure of smooth muscle cells, shows a resemblance with the fast recovery of gap junctional intercellular communication (within 70 min) between C3H/10T1/2 cells exposed to PDGF-BB, as described by these authors.

The gap junctional intercellular communication inhibitory activity of interleukin-6 appeared to be smaller than was observed for PDGF isoforms. In addition, time course experiments demonstrated a slower response of gap junctional intercellular communication inhibition to interleukin-6 treatment. Only when high concentrations (2.5 or 5.0 nM) of interleukin-6 were added to the cells, a quick response of gap junctional intercellular communication inhibition within 1 h of exposure was observed. The slow response of gap junctional intercellular communication inhibition at 0.5 nM interleukin-6 suggests that interleukin-6 may have an 'indirect' effect on gap junctional intercellular communication as well, perhaps by changing the levels of other growth modulating factors in the smooth muscle cell culture. For instance, it is known that interleukin-6 is able to stimulate PDGF production in smooth muscle cells from rat aorta (Ikeda et al., 1991).

In the present study only bFGF stimulated gap junctional intercellular communication between smooth muscle cells. bFGF appeared to be a potent modulator of gap junctional intercellular communication in our study with a doubling of the number of communicating cells after 24 h of incubation with 4.0 nM bFGF. This result is comparable with results of Pepper and Meda (1992) who demonstrated an increase in gap junctional intercellular communication between endothelial cells upon incubation with bFGF.

Our results demonstrate that the modulation of gap junctional intercellular communication between smooth muscle cells is growth factor and cytokine-specific. The reason for the different modulatory effects on gap junctional intercellular communication by growth factors and cytokines is unknown, but may be associated with differences in signal transduction mechanisms. The signal transduction pathway after PDGF receptor activation on smooth muscle cells is relatively well known. Inositol 1,4,5-triphosphate release, diacylglycerol production, calcium mobilization, activation of protein kinase C and changes in intracellular pH have been reported (Block et al., 1989; Inui et al., 1994; Sachinidis et al., 1990), processes which may modulate gap junctional intercellular communication by affecting the permeability of gap junction channels. Pelletier and Boynton (1994) observed that inhibition of gap junctional intercellular communication by PDGF-BB was dissociable from the PDGF receptor tyrosine kinase activity in C3H/10T1/2 cells. Furthermore, they found that PDGF treatment of these cells resulted in phosphorylation of the connexin 43 protein. Growth factors may regulate gap junctional intercellular communication at the level of connexin transcription, mRNA stability, translation and post-translational processing as well: bFGF treatment of endothelial cells resulted in an increase in connexin 43 expression (Pepper and Meda, 1992). Moreover, Kardami et al. (1991) have shown that bFGF-like peptides are an integral part of, or exist in close association with gap junctions and may thus modulate gap junctional intercellular communication.

The biological consequences of the modulation of gap junctional intercellular communication by PDGF-AA, PDGF-BB, interleukin-6, interferon-γ and bFGF are not easily explainable. Extrapolation from these in vitro data to the in vivo situation is difficult, since in the in vitro assay, for instance, the arrangement of smooth muscle cells will deviate from the organization of smooth muscle cells in the vascular wall. Furthermore, primary human smooth muscle cells from umbilical cord arteries were used in our experiments, which may differ from more adult smooth muscle cells in atherosclerotic plaques. Both the percentage of increase or decrease in gap junctional intercellular communication and the duration of the effect caused by these growth modulating factors may be of importance in determining the relevance for the process of atherogenesis. Growth modulating factors like interferon-γ and tumor necrosis factor α, that cause a persistent reduction in gap

junctional intercellular communication, are more likely to have impact on the disturbance of normal smooth muscle cell proliferation than growth factors and cytokines which modulate gap junctional intercellular communication only temporarily. However, growth factors and cytokines that cause only a temporal modulation of gap junctional intercellular communication may be continuously present and the development of atherosclerotic lesions may take many years.

In agreement with many reports in literature, PDGF-AA, PDGF-BB, interleukin-6, interferon-γ, bFGF and tumor necrosis factor α all stimulated smooth muscle cell proliferation in our hands using the MTT test. However, the mitogenic responses of the smooth muscle cells varied considerably in experiments. This heterogeneity in smooth muscle cell response with respect to cell proliferation has been reported before (Absher et al., 1989; Grainger et al., 1994; Hall et al., 1991; Sachinidis et al., 1990; Schwartz et al., 1990; Yamamoto and Yamamoto, 1994). Differences between 'smooth muscle cell batches' may stem from their derivation from different individuals, variations in cell passage numbers and the possible interchange between the synthetic, secretory phenotype in which cells experience growth and proliferation, and the contractile, nonproliferating phenotype. Hall et al. (1991) suggest that the heterogeneity in smooth muscle cell cultures may also represent a heterogeneity of vascular smooth muscle cells in vivo. This heterogeneity in vivo may reflect a specialization of function related to the location of smooth muscle cells in the vascular tissue. These differences in smooth muscle cell batches may result in differences in for instance smooth muscle cell size, saturation density, synthesis of matrix components, cell surface receptor number and proliferation rate. Also, it is not unlikely that cell density influences the smooth muscle cell proliferative response.

Both in vivo and in vitro studies (reviewed by Ruch, 1994) demonstrated cell cycle-related changes in gap junctional intercellular communication. The growth state of cells may influence junctional sensitivity (Paulson et al., 1994). Accordingly, several reports suggest a link between modulation of gap junctional intercellular communication and mitogenesis (reviewed by Trosko et al., 1994). Yamasaki et al. (1993) report a relationship between increased cell proliferation and decreased gap junctional intercellular communication in a liver model. Chandross et al. (1995), however, present data concerning the effect of transforming growth factor β and pituitary extract on gap junctional intercellular communication and cell proliferation of Schwann cells, suggesting that factors which stimulate proliferation simultaneously enhance coupling, whereas factors that inhibit proliferation reduce gap junctional intercellular communication. Our observations suggest a more complex relationship between modulation of gap junctional intercellular communication and cell proliferation, since smooth muscle cell mitogens may either reduce or enhance gap junctional intercellular communication between these cells, depending on the growth factor or cytokine used. Observations in favour of this more complex relationship between gap junctional intercellular communication and mitogenesis were previously reported by Madhukar et al. (1989): epidermal growth factor acted as a mitogen on human keratinocytes while transforming growth factor β seemed to suppress DNA synthesis, although both factors inhibited gap junctional intercellular communication between these cells. The complex nature of the relationship between modulation of gap junctional intercellular communication, cell proliferation and the process of atherosclerosis is further reflected by Rennick et al. (1993) who observed that gap junctions, measured by means of connexin 43 immunolabelling, are numerous between smooth muscle cells of the synthetic phenotype, a state in which smooth muscle cells may proliferate. Only a few gap junctions were found in smooth muscle cells of the contractile phenotype, a state in which cell proliferation does not occur. Recently, Blackburn et al. (1995) observed that early stages of human atherosclerosis are characterized by increased expression of immunodetectable connexin 43 gap junctions in the intima. As the disease progresses, however, the quantity of junctions declines, ultimately to levels below those of the undiseased vessel. Unfortunately, nothing is known yet about the functionality of these immunodetectable gap junction proteins.

In conclusion, the results of our study clearly demonstrate that effects of smooth muscle cell mitogens on gap junctional intercellular communication are not univocal and thus cannot be generalized. Furthermore, the results indicate that it may be useful to separate transient and persistent effects on gap junctional intercellular communication with respect to expected or predicted consequences. In atherosclerotic lesions a mixture of growth factors and cytokines will be present. Therefore, it is interesting to examine the interactive effects of combinations of growth modulating factors on gap junctional intercellular communication between smooth muscle cells, in order to mimic the in vivo situation more adequately. At the moment, this is further investigated in our laboratory.

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