Cytokines induce airway smooth muscle cell hyperresponsiveness to contractile agonists

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A variety of cell types that reside in or infiltrate the inflamed submucosa potentially interact with airway smooth muscle cells and alter myocyte function. Eosinophils, macrophages and, particularly, lymphocytes are postulated as being critical in the initiation and perpetuation of the asthmatic response. One mechanism by which immunocytes exert their effects is the production of proinflammatory mediators that may act directly or indirectly on ASM cells. Several cytokine mRNAs and proteins have been detected within the airways of asthmatic subjects. The precise role of these cytokines in altering myocyte function has recently been the focus of a new line of research. Although cytokines have been reported to inhibit β adrenergic receptor responsiveness in airway smooth muscle cells[1] and alter mitogen induced myocyte growth,[2] this article will review new evidence that suggests that cytokines also modulate contractile agonist induced calcium signalling in human airway smooth muscle cells.

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Effect of cytokines on agonist induced calcium responses

Airway smooth muscle in vivo is either electrically quiescent or generates slow waves and some active tone, but does not generate action potentials under resting conditions or when stimulated by neurotransmitters or autocoids. Excitatory stimulation, whether neural, hormonal, or due to the release of autocoids, results in a graded depolarisation and increase in tone in the muscle. Since excitatory and inhibitory stimuli are imposed on a tissue with little intrinsic contractile tone, the degree of muscle stimulation (and bronchodilation) will be the summated effect of bronchoconstrictor and bronchodilator stimuli.

Activation of an airway smooth muscle cell by a bronchoconstrictor induces a rapid rise in the intracellular concentration of calcium, associated with the release of intracellular calcium stores, to a peak level roughly 10 times higher than the resting level (from 100 nM to more than 1 μM at maximum agonist concentration) as shown in fig 1. Following this peak the calcium level falls but remains raised as long as the contractile stimulus is present. The increased intracellular Ca^{2+} concentration results in the activation of the calcium/ calmodulin sensitive myosin light chain kinase, and the subsequent phosphorylation of the regulatory myosin light chain (MLC_{20}) at Ser 19. Phosphorylation of this residue by myosin ATPase activity initiates crossbridge cycling between myosin and actin. ATP binding, hydrolysis, and ADP release continue as long as MLC_{20} is phosphorylated; dephosphorylation terminates crossbridge cycling and relaxes smooth muscle. In addition to the regulation of tension by intracellular Ca^{2+}, several mechanisms have been advanced to explain a lack of correlation between the intracellular Ca^{2+} concentration and tension between different modes of stimulation. The G protein coupled regulation of myosin phosphatase may be an important mechanism by which "sensitisation" of myosin phosphorylation occurs.[3]

Evidence now suggests that tumour necrosis factor (TNF) and interleukin (IL)-1β, cytokines found in the bronchoalveolar lavage fluid of patients with allergen induced asthma,[4] may directly alter myocyte calcium homeostasis and render airway smooth muscle hyperresponsive to contractile agonists. Since airway smooth muscle is an essential effector cell modulating bronchoconstriction, and since calcium regulates airway smooth muscle cell contraction, interactions between cytokines, such as TNFα and IL-1β released from inflammatory cells, and calcium mobilisation may represent a mechanism underlying bronchial hyperresponsiveness in asthma. Amrani *et al*[5] recently investigated whether TNFα modulated cytosolic calcium responses in airway smooth muscle stimulated with carbachol. Carbachol,[5 6] bradykinin, and thrombin[7] induced increases in intracellular Ca^{2+} concentrations were enhanced by TNFα and IL-1 in human airway smooth muscle. As shown in fig 2, both phases
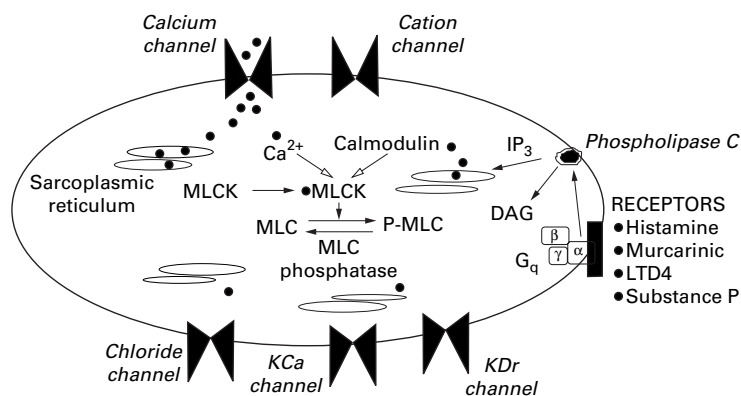

Figure 1 Major signalling molecules associated with contraction and relaxation of airway smooth muscle cells. Transmembrane proteins shown are G protein coupled receptors with associated G protein subunits (α, β, and γ) and plasmalemmal ion channels. A G_q coupled receptor is ligand bound and the associated G protein has undergone nucleotide exchange. The next step will be dissociation of the α and βγ subunits, activation of phospholipase C, and release of intracellular calcium stores. Sources of calcium for contraction are also shown. Calcium permeates voltage dependent calcium channels and non-selective cation channels and is released by the sarcoplasmic reticulum (intracellular structures). Calcium molecules will bind calmodulin and activate myosin light chain kinase (MLCK), which will phosphorylate myosin light chains (MLC). Dephosphorylation occurs by myosin light chain phosphatase, which may be regulated during excitation-contraction coupling. Abbreviations include diacylglycerol (DAG), inositol tris phosphate (IP_3), leukotriene D_4 (LTD_4), substance P (SP) receptor, histamine receptor (H_1), muscarinic receptors (M_3), calcium activated potassium channel (K_{Ca}), and delayed rectifying potassium channel (K_{DR}). Reproduced from reference 47 with permission.
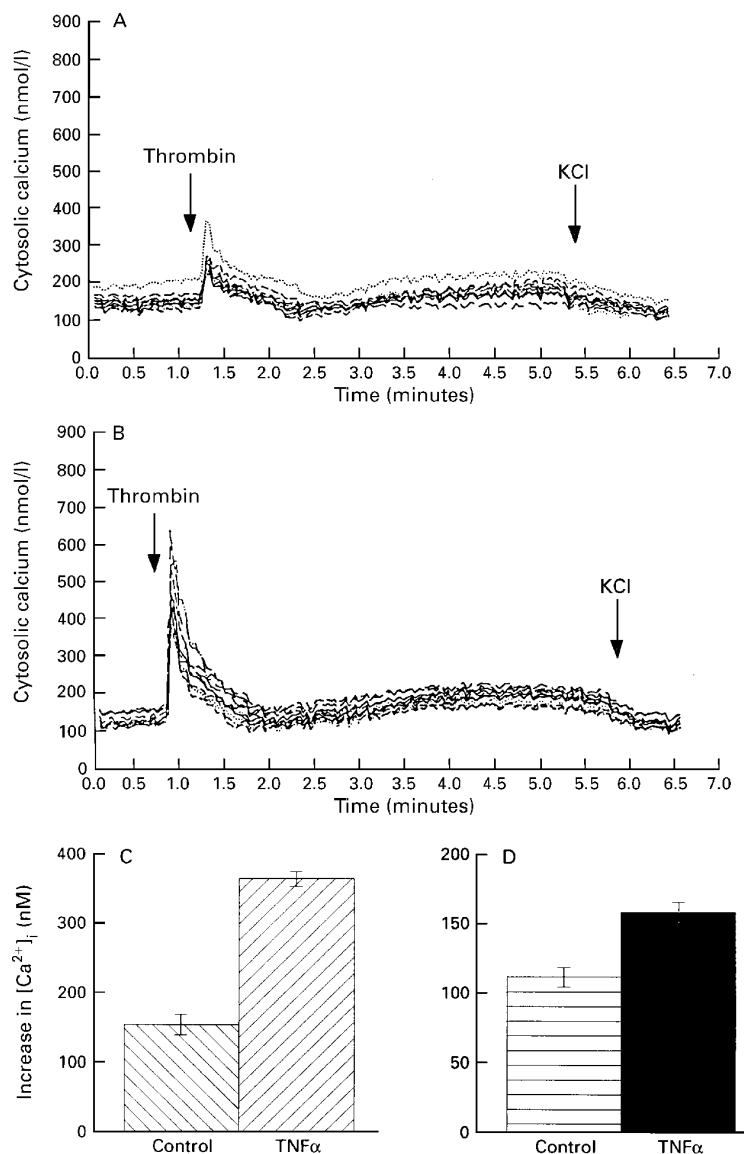
Figure 2 Typical Ca²⁺ traces from cells incubated in the absence (A) or presence (B) of TNFα and respective values for the peak (C) and sustained (D) Ca²⁺ phase from cells incubated in the absence or the presence of TNFα. Results are expressed as the net increase in intracellular concentration of Ca²⁺ over basal (unstimulated) levels. Values are means (SE) of four separate experiments and are significantly different from control (untreated cells; p <0.01). Statistical significance was determined using the unpaired Student's t test. Reproduced from reference 7 with permission.

of thrombin induced calcium levels were affected by TNFα, suggesting increased calcium mobilisation both from intracellular and extracellular stores.[7] Activation of TNFRp55, the predominant receptor expressed in human airway smooth muscle cells, and de novo protein synthesis were also found to be required for the effects of TNFα on calcium mobilisation.[6 7] The ability of TNFα to alter the intracellular Ca²⁺ signals induced by a variety of different agonists suggested that this cytokine may "prime" airway smooth muscle cells for an increase in agonist responsiveness. This is an interesting finding since TNFα can also induce the "primed" airway smooth muscle to become more contractile to the same agonists either in vivo[8–10] or in vitro.[11 12]

Whether both the phenomena of increased receptor mediated calcium responses and increased smooth muscle contractility are linked remains to be determined. However, the ability of IL-1β also to induce bronchial hyperresponsiveness[12] in a similar manner to that of TNFα[7] suggests that cytokines may directly modulate airway myocyte calcium signalling and render the cell hyperresponsive to bronchoconstrictors. Further studies are needed to address whether this potential mechanism may induce bronchial hyperresponsiveness in asthma.

Potential intracellular mechanisms altered by cytokines

G PROTEIN MEDIATED SIGNAL TRANSDUCTION

Since TNFα alone did not stimulate either a calcium response or phosphoinositide hydrolysis in human airway smooth muscle, TNFα probably augments agonist induced increases in intracellular Ca²⁺ by directly affecting the coupling process of agonist receptors to downstream signalling events as shown in fig 3. In human airway smooth muscle such receptors are known to be coupled to phospholipase C which catalyses the hydrolysis of phosphotidylinositol 4,5 bisphosphate, yielding inositol trisphosphate and diacylglycerol.[13 14] Recent studies suggest that TNFα significantly enhances phosphoinositide accumulation in response to bradykinin in human airway smooth muscle and epidermoid carcinoma cells.[7 15] In addition, G protein induced activation of adenylyl cyclase by isoproterenol or G protein mediated arachidonic acid metabolism by bradykinin was also shown to be enhanced by TNFα.[16 17] In airway smooth muscle, however, TNFα as well as IL-1β have been reported to inhibit isoproterenol stimulated activation of adenylyl cyclase.[1 18]

Cytokines have also been reported to modulate expression of G proteins. TNFα increases the amount, as well as the activity, of G proteins in several cell types including airway smooth muscle.[17 19–21] The finding that TNFα enhances calcium mobilisation in response to NaF,[7] an agent that bypasses membrane receptors and directly activates G proteins,[22 23] supports the notion that TNFα acts directly at the level of G proteins rather than modifying the expression of contractile agonist receptors. This observation supports previous findings that, despite increased calcium signals to carbachol, TNFα did not increase muscarinic receptor numbers.[5] Finally, TNFα may also alter phospholipase C activity, as has been shown in a mouse fibrosarcoma cell line.[24] Taken together, these studies show that cytokines modulate airway smooth muscle functions, not only by activating specific cytokine receptors, but also by dramatically amplifying the ability of other agonists to induce increases in the intracellular concentration of Ca²⁺.

INTRACELLULAR CALCIUM STORES

In many cell types the activation of calcium pumps directly regulates the calcium ion concentration both in the cytosol and in intracellular stores, either by extruding calcium
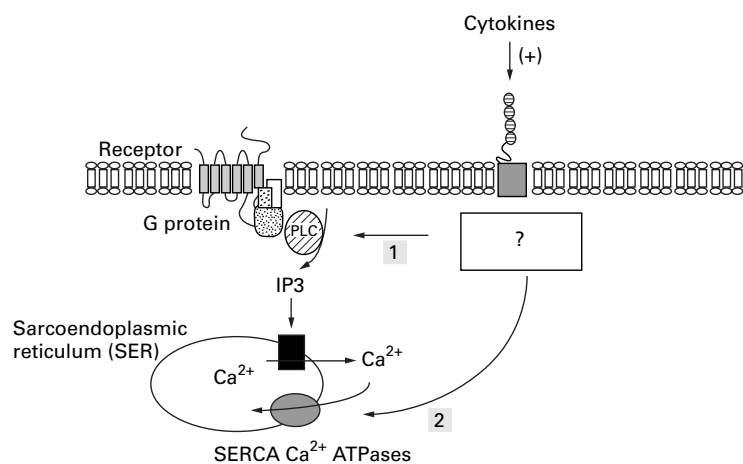
Figure 3 Potential sites for cytokine induced alterations in calcium homeostasis in human airway smooth muscle cells. This involves (1) the receptor/G protein/effector system and (2) the calcium regulatory proteins such as SERCA-type calcium pumps which function by pumping calcium from the cytosol to the internal stores. IP3 = inositol-1,4,5 trisphosphate; PLC = phospholipase C.

from the cell via plasma membrane Ca^{2+}-ATPases[25 26] or by sequestering calcium into intracellular calcium stores by the SERCA-type Ca^{2+}-ATPases.[27–32] A variety of SERCA isotypes (Sarco-Endoplasmic Reticulum Calcium-ATPase) are expressed in different tissues and are the products of both distinct genes and alternative mRNA splicing. For the SERCA-type Ca^{2+}-ATPases, three genes have been identified: $SERCA_1$, expressed in fast skeletal muscle[33]; $SERCA_2$ which gives rise to $SERCA_{2a}$ and $SERCA_{2b}$ isoforms,[27 30] mainly expressed in cardiac and smooth muscle, respectively; and $SERCA_3$ which is a non-muscle isoform.[27]

Thapsigargin, a specific inhibitor of SERCA,[34–36] has been useful in defining the role of SERCA associated calcium pools in activating cellular signal transduction pathways. Thapsigargin sensitive calcium stores not only provide a source of calcium following bronchoconstrictor stimulation[31 32] but also appear to exert a profound control over cell proliferation and progression through the cell cycle.[37 38] Calcium responses to thapsigargin, which triggers calcium signals by directly releasing intracellular stores, are potentiated by pretreating cells with TNFα.[6 39] This effect may be due to an increased release of calcium, to an increase in calcium content, or to an increase in the number of SERCA-type calcium-ATPases sensitive to thapsigargin. Alternatively, cytokines may directly affect the intracellular Ca^{2+} concentration by altering the type of SERCA associated with the calcium stores. Interestingly, $SERCA_{2a}$ protein and mRNA content are increased in a time dependent manner by TNFα.[40] Several reports have also shown a modulation of SERCA-type Ca^{2+}-ATPase expression in pathological conditions such as hyperthyroidism and hypertension.[41–44] In other studies thyroid hormone,[41] platelet-derived growth factor,[45] and insulin growth factor[46] upregulated transcription of the $SERCA_2$ and $SERCA_1$ gene. Taken together, these studies suggest that calcium pumps that regulate intracellular calcium stores may also be altered

by cytokines and growth factors and may represent another pathway by which cytokines alter agonist induced calcium responses or modulate cell growth.

Summary
The important pathophysiological features of the airways in asthma include exaggerated narrowing to bronchoconstrictor agonists and attenuated relaxation to β adrenoceptor stimulation. These physiological perturbations are associated with inflammation and remodelling of the airways, the latter including an increase in airway smooth muscle cell mass, disruption of the airway epithelium, and changes in the airway tissue extracellular matrix. Recent evidence suggests that cytokines, important molecules modulating airway inflammation, also directly decrease airway smooth muscle responsiveness to β adrenergic agents, stimulate cytokine secretion, inhibit or promote airway smooth muscle proliferation, and "prime" airway smooth muscle to become hyperresponsive to bronchoconstrictors. Characterisation of the cellular and biochemical events that are involved in activation of airway smooth muscle is likely to be the major consideration in the design of future therapies for asthma. Because calcium is an essential regulatory element for cell growth and cell contraction, it is likely that alterations in calcium mobilisation may, in part, play a role in creating an airway smooth muscle phenotype that is hyperresponsive to contractile agonists. Further studies will be required to determine the precise mechanisms involved in cytokine modulation of calcium homeostasis in airway smooth muscle.

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