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NeuroToxicology 22 (2001) 795–810

NeuroToxicology

Neurogenic Inflammation and Particulate Matter (PM) Air Pollutants☆

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Received 29 June 2001; accepted 20 August 2001

Abstract

Exposure to a class of airborne pollutants known as particulate matter (PM) is an environmental health risk of global proportions. PM is thought to initiate and/or exacerbate respiratory disorders, such as asthma and airway hyper-responsiveness and is epidemiologically associated with causing death in the elderly and those with pre-existing respiratory or cardiopulmonary disease. Plausible mechanisms of action to explain PM inflammation and its susceptible sub-population component are lacking. This review describes a series of published studies which indicate that PM initiates airway inflammation through sensory neural pathways, specifically by activation of capsaicin-sensitive vanilloid (e.g. VR1) irritant receptors. These acid-sensitive receptors are located on the sensory C nerve fibers that innervate the airways as well as on various immune and non-immune airway target cells. The activation of these receptors results in the release of neuropeptides from the sensory terminals that innervate the airways. Their interactions with airway target cells, result in signs of inflammation (e.g. bronchoconstriction, vasodilation, histamine release, mucous secretion etc.). Our data have linked the activation of the VR1 receptors to the surface charge carried on the colloidal particulates which constitute PM pollution. Related studies have examined how genetic and non-genetic factors modify the sensitivity of these irritant receptors and enhance the inflammatory responsiveness to PM. In summary, this review proposes a mechanism by which neurogenic elements initiate and sustain PM-mediated airway inflammation. Although neurogenic influences have been appreciated in normal airway homeostasis, they have not, until now, been associated with PM toxicity. The sensitivity of the sensory nervous system to irritants and its interactions with pulmonary target tissues, should encourage neuroscientists to explore the relevance of neurogenic influences to toxic disorders involving other peripheral target systems.

Keywords: Particulate matter; Neurogenic inflammation; Tracheal epithelial cells; BEAS-2B; Somatosensory neurons; Vanilloid receptors; Capsaicin receptors; Acid-sensitive pathways

INTRODUCTION AND OVERVIEW

Exposure to airborne particulate matter (PM) air pollution is epidemiologically associated with mortality and morbidity in the international and national community. A mechanism of action to explain these consequences of PM exposure is lacking. Our research has demonstrated that PM initiates inflammation in airway target cells through a phenomenon known as neurogenic inflammation (NGI). This pathophysiology involves the activation of irritant receptors (vanilloid, VR1) and acid-sensitive pathways. Subsequently, pro-inflammatory neuropeptides and cytokines are released from these airway target cells and stimulate cellular expressions of airway inflammation. The discovery of these acid sensitive receptors on human respiratory epithelial cells and a description of their response to PM exposure are described in the following sections. More recent studies have addressed the

☆This manuscript has been reviewed by the National Health Effects and Environmental Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC and approved for publication. Approval does not signify that the contents necessarily reflect the view and policies of the Agency, endorsement, or recommendation for use.

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0161-813X/01/$ – see front matter
PII: S0161-813X(01)00062-6

physicochemical features of PM that are most culpable in triggering these receptors. Based on these findings, a neurogenic hypothesis has been developed suggesting that the surface charge of PM initiates airway inflammation by activation of vanilloid (i.e. capsaicin, VR1) receptors and acid-sensitive pathways. A current area of research examines the ''sensitive sub-population'' component of PM exposure, which renders the young, the old, and those with pre-existing cardio-pulmonary conditions especially vulnerable. This paper first provides an overview of the sensory nervous system and its role in the phenomenon of neurogenic inflammation and toxicant-induced airway disorders. A chronology of studies that have contributed to a neurogenic explanation of PM airway toxicity, follows. PM generated from industrial emissions (residual oil fly ash, coal fly ash, oil fly ash), volcanic (Mt. St. Helen), residential (woodstove) and urban ambient (St. Louis, Ottawa), were exposed to mice or cell culture models (tracheal epithelial cells, dorsal root and trigeminal ganglia neurons) of airway-relevant targets. Endpoints included single cell and population calcium recordings, RT-PCR, measures of inflammatory cytokine release, cobalt histochemistry and morphometry. The studies described in this review propose a mechanism by which neurogenic elements initiate and sustain PM-mediated airway inflammation. Such neurogenic influences have been appreciated in normal airway homeostasis, but have not, until now, been associated with PM inflammation nor been implicated in its associated morbidity.

Sensory Innervation of the Airways

The nasal and pulmonary airways are innervated by central and peripheral branches of the cholinergic, noradrenergic, and adrenergic neural pathways (Barnes, 1986a, 1990; Widdicombe, 1986; de Jongste et al., 1991; Lundberg, 1995). Together and singly, they act to regulate respiratory rhythm by controlling ventilation. In addition to these classic innervation pathways, there are those, whose effects are not blocked by cholinergic or adrenergic antagonists. This ''non-adrenergic–non-cholinergic (NANC) innervation system, is largely sensory and derives from parasympathetic and sympathetic neurons that are located in various ganglia found along the brain stem, spinal cord and the tracheal walls (Lammers et al., 1992). Fibers from the trigeminal, dorsal root, nodose, and jugular ganglia project their fibers to the nasal, upper and lower airways. These nerve fibers are bipolar, with one axon terminating in the spinal cord or brain and the other synapsing in peripheral tissues (e.g. skin, gut and the airways) where exogenous elements are most likely to enter the body (i.e. ''port of entry'' tissues). In such tissues, sensory fibers can have several types of nerve terminals. Elaborate polymodal receptors (e.g. Meisner's, Pacinian) which are responsive to mechanical, vibratory and tactile stimuli, are most often found in dermal and intestinal tissues. In contrast to these, free nerve endings, lacking morphologically distinct terminals, characterize the sensory C fibers which innervate the airway lumen and the interstitial tissues. These free nerve endings form a structural and functional relationship with a variety of non-neuronal target cells (e.g. epithelial cells, muscle cells, mast cells and macrophages). Irritant receptors, located on the terminals of sensory fibers, are activated by noxious stimuli and modulated by endogenous inflammatory agents (e.g. bradykinin, prostaglandins, histamine). Upon their activation, a variety of neuropeptide transmitters such as substance P (SP), calcitonin gene related protein (CGRP), and neurokinin A (NKA), are released. These neurotransmitters are potent inducers of a cascade of airway inflammatory responses that include vasodilatation, mucous secretion, plasma protein extravasation, leukocyte adhesion-activation, and bronchoconstriction. These responses are caused by the interactions of peptides with immune cells and non-immune cells found throughout the ''port of entry'' tissues, alluded to earlier (e.g. conjunctiva, mucous membranes, skin, gut, airways). Such interactions result in the initiation of a rapid, protective response to foreign or adverse stimuli, known as 'neurogenic inflammation'. In this process, a cascade of cellular and sub-cellular events occurs, culminating in the more overt symptoms of tissue inflammation (i.e. tenderness, swelling and reddening). Historically, these symptoms have been described in vascular and immunological terms, however, more current thinking indicates that the sensory nervous system and its peptidenergic transmitters (i.e. SP, CGRP, NKA) initiate and sustain this inflammation (Barnes, 1986a, 1986b; McDonald, 1988; Solway and Leff, 1991; Meggs, 1993; Maggi, 1993; Lundberg, 1995; Baluk, 1997; Di Maria et al., 1998; Baluk et al., 1999; Joos et al., 2000). Once released, these pro-inflammatory peptides interact with a variety of immune (e.g. lymphocytes, neutrophils, macrophages, eosinophils) and non-immune (e.g. smooth muscle, endothelia cells of the vasculature, epithelial cells that line the lumen of the airways and gut, keratinocytes of the skin) target cells. In the airways, the effects of tachykinin neuropeptides are largely mediated by NK1 and NK2 receptors found on non-neural target cells.

NKA, acting on NK2 receptors, stimulates smooth muscle contraction, while the vascular and more inflammatory effects are mediated by SP acting on NK1 receptors. NGI is often independent of higher nervous system function (i.e. central nervous system) and produces many of the overt symptoms of inflammation (e.g. erythema, edema, vasodilation, vasoconstriction, mucous secretion) through the phenomenon of the ''axon reflex'' (Maggi, 1993; Lundberg, 1995). Upon involvement of the immune system, the initial symptoms of inflammation are exacerbated and perpetuated, with resulting tissue damage (Joos et al., 2000).

Sensory Irritant Receptors

The sensory nervous system is especially relevant to chemically-induced airway inflammation since it functions to protect against noxious and potentially tissue-damaging stimuli. Receptors (e.g. capsaicin, acid-sensitive, mechanoreceptors) found along the distal ends of these sensory C fibers, are activated by a wide variety of irritants such as acid, chemical irritants, heat, cold, pressure and the botanical toxin, capsaicin. Upon their activation, a rapid influx of ions (e.g. calcium, sodium) from extracellular stores, enters the cells and stimulates the release of inflammatory neuropeptides from the distal ends of the sensory fiber (Wood and Docherty, 1997). There is an inherent age-dependent variability in the number and distribution of the irritant-sensitive receptors among species and within different tissues of the same species (Kirischuk et al., 1992; Szallasi, 1994; Szallasi and Blumberg, 1993; Acs and Blumberg, 1994). Even within the same species and cell-type (e.g. dorsal root sensory neurons), variants of the capsaicin-sensitive (vanilloid or VR1) irritant receptors have been identified (Caterina et al., 1999).

Irritant receptors and neuropeptide-release are not proprietary to sensory nerve fibers. Various types of respiratory epithelial cells (e.g. neuroendocrine cells, Clara cells, and type II alveolar cells) have been shown to contain neuropeptide granules and neuropeptide receptors and have been shown to respond to neuropeptide exposure with cytokine release (Baluk et al., 1993; Mullol et al., 1997; Veronesi et al., 1999a; Stevens et al., 1997). Capsaicin-sensitivity and functional capsaicin receptors have been identified on other airway-relevant immune cells (i.e. alveolar macrophages, monocytes, RAW mono/macrophage cells) (Colquhoun et al., 1995; Garle et al., 2000; Baluk et al., 1993; Hastings and Hua, 1995; Savitha et al., 1990). In humans, inflammatory cells such as eosinophils, macrophages, epithelial cells, lymphocytes, and dendritic cells can themselves produce and release pro-inflammatory peptides (i.e. SP, NKA) upon irritant exposure (Joos et al., 2000). These findings indicate that, in addition to sensory neurons and their terminals, a variety of airway relevant cells contain functional irritant receptors that, when triggered by noxious stimuli (e.g. capsaicin, acid-pH, chemical irritants), release neuropeptides.

Neurogenic Inflammation and Environmentally-Relevant Airway Disorders

An impressive body of clinical and experimental literature links the hyper-vigilance of the sensory nervous system with neurogenic inflammation (NGI) in the airways (Barnes, 1986b, 1991; Meggs, 1993; Maggi, 1993; Lundberg, 1995; Widdicombe, 1996a; Baluk, 1997). The sensory nervous system and NGI is associated with the chronic inflammation and airway remodeling reported in asthmatics (Barnes, 1999; Holt et al., 1999) and that associated with respiratory irritants such as ozone (Gordon et al., 1985; Yeadon et al., 1992; Joad et al., 1996; Albright and Goldstein, 1996), sulfur dioxide (Koenig and Pierson, 1991) (el-Fawal et al., 1995), hydrogen sulfide (Prior et al., 1990), acrolein (Springall et al., 1990) and toluene diisocyanate (Sheppard and Scypinski, 1988; Gagnaire et al., 1997). Disease states such as asthma or viral infections are often marked by damage to the epithelial cell population which lines the nasal and pulmonary lumen. In such conditions, an up-regulation of neuropeptide NK1 receptors and increased sensory nerve excitability have been observed (Lundberg, 1995; Joos et al., 2000; Barnes, 1991).

The following studies implicate the sensory nervous system and NGI with the airway inflammation associated with exposure to particulate matter, a class of airborne pollutants. To demonstrate this relationship, multidisciplinary endpoints have been used that include airway hyper-responsiveness and bronchial lavage in animals and histochemical, immunocytological, biophysical, and molecular endpoints in cell culture models of sensory neurons that innervate the airways and their epithelial target cells. In these studies, PM derived from various industrial, urban ambient, industrial, residential, and volcanic sources have been used (see Table 1).

PARTICULATE MATTER (PM)

Both national and international epidemiological studies indicate a significant association between increases

Table 1
Particulate matter air pollutants

Residual oil fly ash (ROFA)	Collected on a Teflon glass filter from an industrial power plant (Southern Research Institute, Birmingham, AL)
Woodstove	Particulates collected (i.e. scraped) from the exhaust pipes of a commercially available, residential woodstove in Durham, NC
Mt. St. Helen (MSH)	Volcanic dust collected from St. Helen in 1980 near Ritzville, WA
Ottawa	Urban ambient EHC-93 from Environmental Health Canada collected in bag-house in 1993
St. Louis	Urban ambient[a] SRM 1648
Coal fly ash (CFA)	Collected on glass filters from a Western US conventional coal burning power plant
Oil fly ash (OFA)	Industrial PM collected on Teflon-coated fiber glass filter (250–300 °C) from the low sulfur, residual oil burning in a Niagara power plant

[a] Standard reference materials (SRMs) (Huggins et al., 2000) from the National Institute of Standards (Washington, DC) are shown. These PM were obtained from the Human Studies Division of the US Environmental Protection Agency, Chapel Hill, NC.

in morbidity and mortality and short-term increases in the level of the ambient PM (Samet et al., 2000). The morbidity (i.e. asthma, bronchitis, airway hyperresponsiveness) and mortality associated with PM exposure are marked by a sensitive sub-population component, characterized by increased sensitivity in the young and old, asthmatics, smokers and in those with pre-existing respiratory or cardiopulmonary conditions (Pope, 2000; Utell and Frampton, 2000). While the epidemiological association between PM pollutants and adverse health effects is clear (Dockery et al., 1992; Dockery and Pope, 1994; Schwartz, 1994, 1996; Pope, 1996, 2000; Burnett et al., 1998; Liao et al., 1999; Hoek et al., 2000), mechanisms of action to explain ambient PM toxicity and its sensitive sub-population phenomenon are lacking.

PM comes from diverse natural (sea spray, volcanic, earth erosion) and anthropogenic (industrial, urban, residential, environmental, traffic exhaust) sources and consists of complex aggregates of elemental and organic carbons, volatile organics, metals, sulfates, pesticides, pollen, microbial contaminants and unknown materials, complexed to an insoluble carbon core. This complexity has hampered investigations into the culpable factor(s) of PM toxicity and suggests that PM may target a variety of pathways. Consequently, most experimental research has focused on the individual chemical components identified on certain industrial PM such as transition metals (Hatch et al., 1985; Gilmour et al., 1996; Pritchard et al., 1996; Dreher et al., 1997; Carter et al., 1997; Gavett et al., 1997; Kadiiska et al., 1997; Dreher et al., 1998), biologicals (e.g. endotoxins, pollen) (Becker et al., 1996), and acidic components (Chen et al., 1992; Brauer et al., 1995; Lippmann and Thurston, 1996; Kimmel et al., 1997; Zelikoff et al., 1997; Gwynn et al., 2000). In spite of the physicochemical complexity of PM, however, the levels of mortality and morbidity expressed in exposed populations are remarkably uniform across the various geographic locations in which the epidemiological studies are performed (Schwartz, 1994; Utell and Frampton, 2000). This uniformity is preserved even when epidemiological data from areas with widely varying emission sources are compared (Utell et al., 1991; Dockery and Pope, 1994; Finlayson-Pitts and Pitts, 1997; Samet et al., 2000). This relationship suggests that PM may be affecting toxicity through a more common physicochemical mechanism.

EXPERIMENTAL CHRONOLOGY

Respiratory Epithelial Cells Contain Sensory Receptors

Sensory C nerve fiber terminals and their epithelial target cells constitute the initial cellular unit to encounter airborne pollutants. Unfortunately, their interactions in response to chemical irritants and the subsequent inflammatory cascade, are poorly understood. Consequently, these interrelationships were examined using a human, immortalized, tracheal–bronchial epithelial cell line (BEAS-2B) (Reddel et al., 1988). These respiratory target cells have been featured in numerous studies involving airborne pollutants (Samet et al., 1992; McKinnon et al., 1993; Carter et al., 1997), and show inflammatory responses remarkably similar to human primary epithelial cultures.

BEAS-2B cells were first examined for their response to physiological concentrations of SP, acid (pH 6.5–5.0), or the biological irritant, capsaicin. Exposure of SP resulted in immediate increases in intracellular calcium concentration (i.e. $[Ca^{2+}]_i$), the synthesis 2 h later of transcripts for the pro-inflammatory cytokines, IL-6, IL-8 and TNFα, and the subsequent release

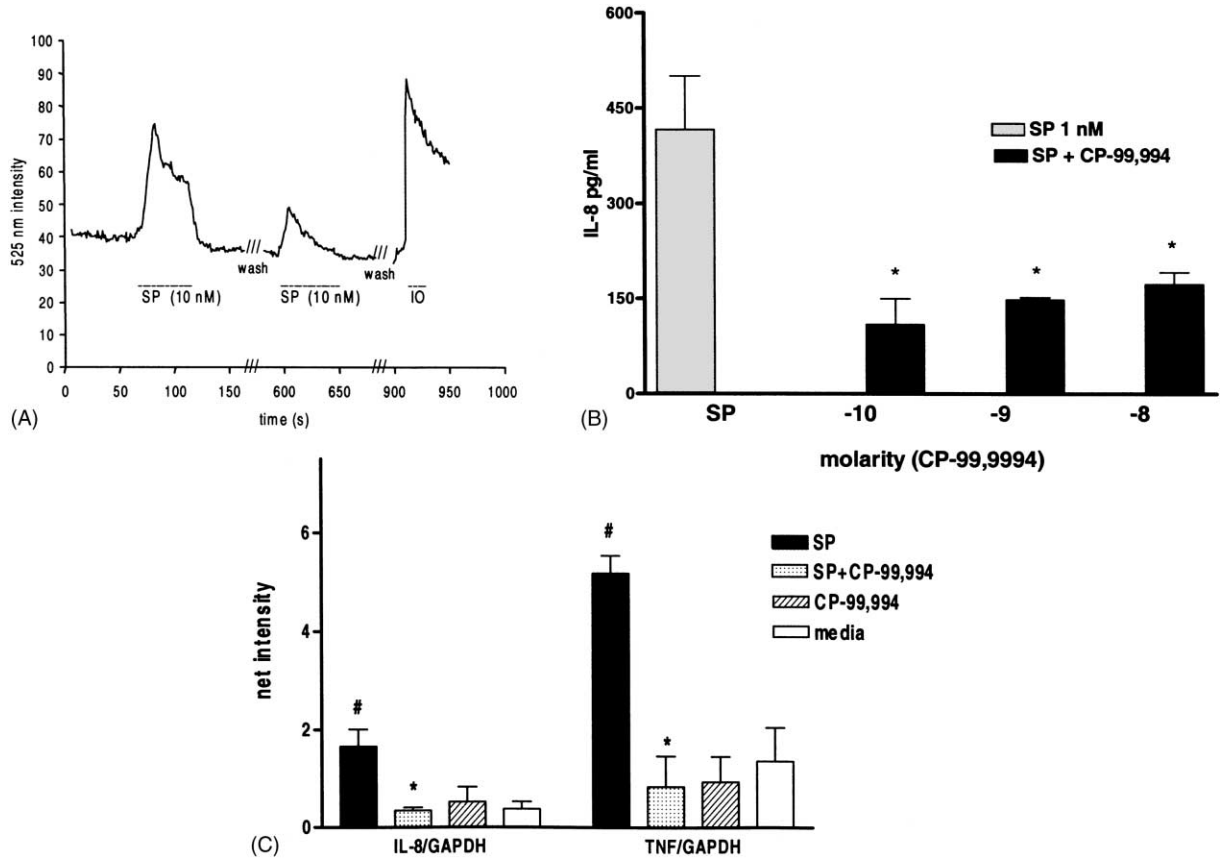

Fig. 1. (A) Increases in $[Ca^{2+}]_i$ levels were measured in BEAS-2B cells using the fluorescent probe Fluo-3 AM. Exposure to SP (10 nM) produced a rapid increase in $[Ca^{2+}]_i$, followed by a lower, longer lasting plateau. A second exposure of the cells to SP produced a $[Ca^{2+}]_i$ response with a smaller peak amplitude, suggesting receptor desensitization or internalization. IO (2 μM) was applied at the end of each experiment to determine the maximal $[Ca^{2+}]_i$ increase. (B) BEAS-2B cells were also pretreated with 0.1–10 nM CP-99,994, a non-peptide, competitive antagonist of the NK1 receptor before exposure to SP (1 nM). IL-8 release was significantly reduced at all concentrations of the antagonist. Media values are subtracted from all treatment groups. Significance ($P < 0.05$) from controls is denoted by (∗). (C) RT-PCR data on inflammatory cytokine transcript levels indicated that transcripts for IL-8, and TNFα were significantly depressed in cultures treated with CP-99,994 (1 nM) before a 2 h exposure to SP (10 μM, 1 nM). The symbol (#) indicates significance ($P < 0.05$) from media controls and asterisks (∗) indicate significance ($P < 0.05$) from SP-treated cells. Reproduced with permission (Veronesi et al., 1999a).

of these cytokines, 4 h post-exposure. Moreover, this release occurred in a receptor-mediated fashion, since cytokine transcripts and their proteins were reduced by pretreatment with antagonists to NK1 receptors (Fig. 1). Acid pH and capsaicin also stimulated increases in $[Ca^{2+}]_i$ and the release of inflammatory cytokines (Fig. 2), changes which were partially inhibited by amiloride and by the capsaicin receptor antagonist, capsazepine (CPZ) (Bevan et al., 1992). BEAS-2B cells, identified as capsaicin or acid-sensitive by a cobalt-based histochemical technique (Wood et al., 1988), were morphometrically analyzed to show a concentration-dependent response of the cobalt precipitate in response to both acid pH and capsaicin. Together, these studies (Veronesi et al., 1999a) indicated that human tracheal–bronchial epithelial cells (i.e. BEAS-2B) housed irritant receptors and acid-sensitive pathways, which when triggered, released inflammatory cytokines.

Role of VR1 Receptors in ROFA Inflammation

To address the possibility that PM could initiate inflammation through a NGI mechanism, residual oil fly ash (ROFA), an industrial emission source PM pollutant, was first tested. ROFA is generated from the burning of low sulfur residual oil, and is composed primarily of highly soluble transition metals and sulfates (Henry and Knapp, 1980; Pritchard et al., 1996; Dreher et al., 1997). Although ROFA is atypical relative to ambient PM in terms of its chemical make-up and in the level of its associated inflammation in experimental models, it contains components (i.e. metals, sulfates, acids) that could be relevant to the

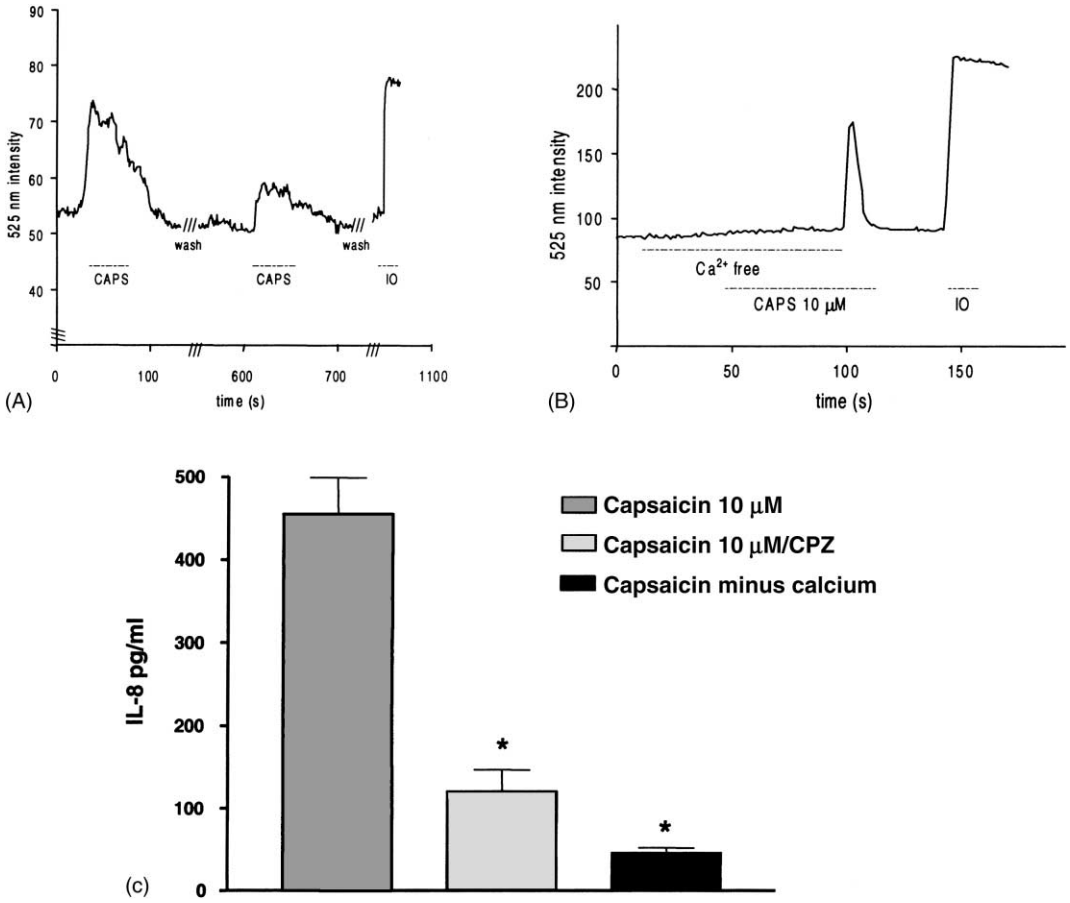

Fig. 2. (A) Cells, exposed to 10 μM capsaicin (CAPS) produced a rapid increase in $[Ca^{2+}]_i$ that declined over time. After washing, a second exposure to CAPS (10 μM) increased $[Ca^{2+}]_i$ but with a reduced response amplitude, suggesting receptor desensitization. (B) Increases of $[Ca^{2+}]_i$ did not occur when cells were bathed in calcium–magnesium-free HEPES buffer. When the wash solution was changed to one containing CAPS (10 μM) in calcium–magnesium media, an immediate increase in $[Ca^{2+}]_i$ occurred. (C) Capsaicin (10 μM) stimulated IL-8 release after 4 h exposure. This release was significantly (∗) reduced (>75%) by pre-exposure to CPZ (12 mM), an antagonist of VR1 receptor(s). BEAS-2B cells, exposed to capsaicin in calcium–magnesium-free media, failed to release IL-8. Media values were subtracted from all treatment groups. Reproduced with permission (Veronesi et al., 1999a).

underlying mechanisms of PM inflammation. Tracheal instillation of ROFA in mice and rats produces airway hyper-responsiveness and acute lung injury, consisting of epithelial damage, pulmonary edema, hemorrhage, and influx of neutrophils, macrophages, and eosinophils (Su et al., 1995; Dreher et al., 1997; Dye et al., 1997; Kodavanti et al., 1997a). ROFA also produces a species-dependent oxidative burst, inflammatory cytokine release and apoptosis when exposed to cultures of alveolar macrophages (Becker et al., 1996; Rahman et al., 1997; Holian et al., 1998). Human primary bronchial epithelial cells and the immortalized BEAS-2B cells, release various cytokines (i.e. IL-6, IL-8, TNFα) and inflammatory mediators in response to ROFA (Carter et al., 1997). Sub-cellular changes associated with ROFA exposure include alterations in signal transduction (Samet et al., 1997; Baeza-Squiban et al., 1999), and free radical formation due to its high transition metal content (Li et al., 1996; Dye et al., 1997).

Since we had shown that respiratory epithelial cells contained both neuropeptide and acid-sensitive irritant receptors and pathways (Veronesi et al., 1999a), their role in the cellular inflammation associated with ROFA exposure was examined (Fig. 3). ROFA-exposed BEAS-2B cells responded with an immediate increase in $[Ca^{2+}]_i$ followed by a concentration-dependent release of IL-6. To test the relevance of neuropeptide or capsaicin receptors to these changes, BEAS-2B cells were pretreated with neuropeptide receptor antagonists (CP-99,994, CGRP 8–32) or CPZ, the antagonist for the capsaicin (i.e. VR1) receptor. Although antagonists to the NK1 and CGRP receptors reduced ROFA-stimulated IL-6 cytokine production by ∼25 and 50%, pretreatment of cells with CPZ inhibited the immediate increases in $[Ca^{2+}]_i$, diminished transcript (i.e. IL-6,

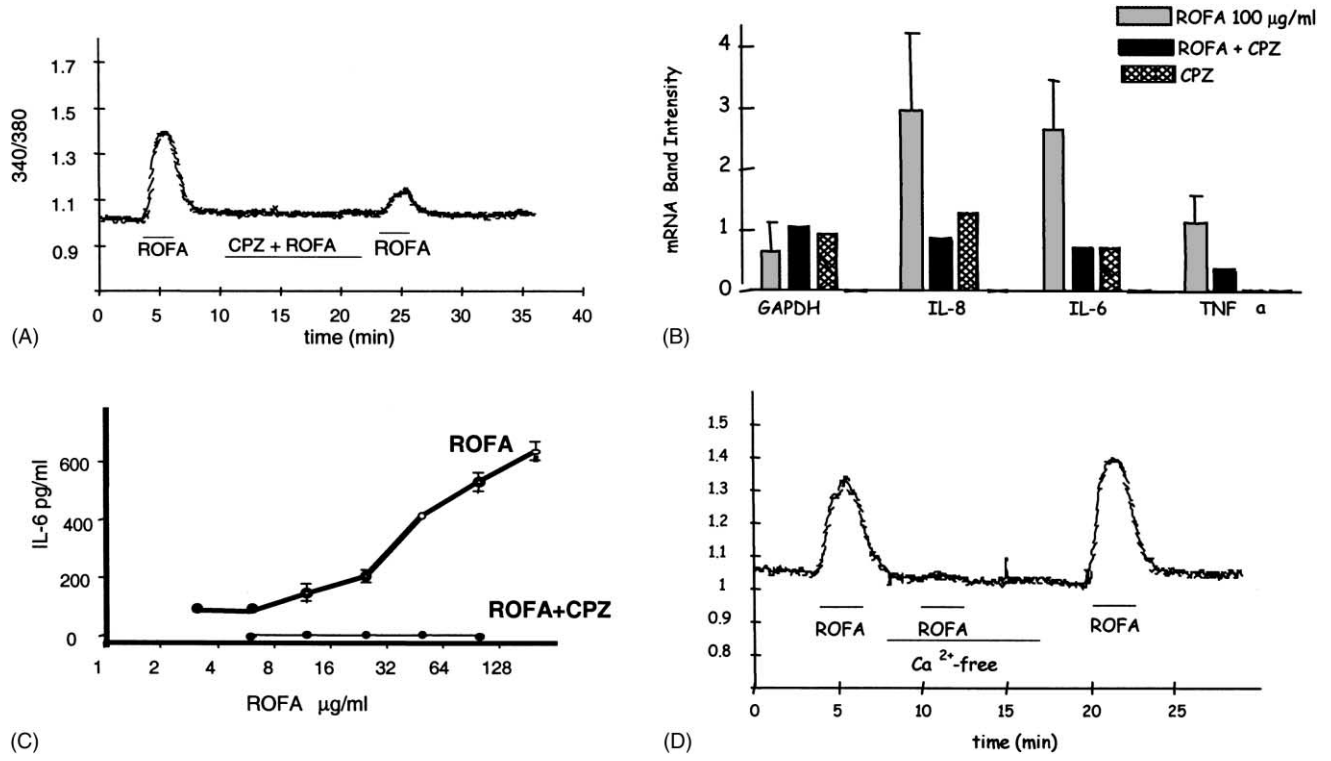

Fig. 3. Pretreatment of BEAS-2B with CPZ (5–20 μM), the antagonist to the VR1 capsaicin receptor, (A) inhibited the ROFA-induced increase in $[Ca^{2+}]_I$, (B) reduced pro-inflammatory transcripts for IL-8, IL-6 and TNFα cytokines; and (C) reduced the IL-6 release in BEAS-2B cells to control levels. (D) The ROFA response was also abolished in the absence of extracellular calcium. Reproduced with permission (Veronesi et al., 1999b).

IL-8, TNFα) levels and reduced IL-6 cytokine release to control levels. In addition, these experiments showed that such changes were dependent on extracellular calcium, a characteristic of capsaicin-sensitive receptors (Winter et al., 1993; Kuenzi and Dale, 1996).

ROFA, and other PM stimulate a variable release of inflammatory cytokines and an oxidative burst and apoptosis in human alveolar macrophages (HAM) (Becker et al., 1996). HAM, pre-exposed to CPZ (15 min) before ROFA exposure, prevented this oxidative burst (as measured by chemiluninescence) (Mudpalli, 1999). Subsequent studies indicated that apoptosis could also be blocked in ROFA-exposed HAM or in a mouse macrophage/monocyte cell line (i.e. RAW 254) that had been pretreated with a variety of non-cytotoxic concentrations CPZ (Becker, and Veronesi, unpublished data).

The inflammation and airway hyper-responsiveness associated with a variety of air pollutants are reduced in animals whose sensory fibers have been destroyed by capsaicin-treatments or interrupted with antagonists to neuropeptide or capsaicin receptors (Prior et al., 1990; Nielsen, 1991; Yeadon et al., 1992; Satoh et al., 1993; Scheerens et al., 1996). The relevance of sensory innervation and its irritant receptors to ROFA inflammation was subsequently examined in a murine model of airway hyper-responsiveness. BALB/c mice were denervated of polymodal sensory C fibers by neonatal capsaicin treatment (Hayes et al., 1981) and as adults administered ROFA by tracheal instillation. These ''sensory denervated'' animals showed a significant reduction in airway hyper-responsiveness, inflammatory cell (neutrophils) influx and LDH release relative to normal mice exposed to ROFA (Gavett et al., 1998). To replicate this phenomenon in culture, sensory ganglia cultured from these denervated animals, failed to release cytokines in response to a variety of irritants and PM (Fig. 4).

Taken collectively, these data indicated that the capsaicin-sensitive sensory system and its associated acid-sensitive irritant receptors played an initiating role in ROFA inflammation in two critical human airway target cells (i.e. tracheal epithelial cell, alveolar macrophage) and in whole animals.

ROFA "Surrogates"

PM is marked by an extreme heterogeneity in its physicochemical make-up, a complexity which has resulted in a variety of hypotheses that associate the

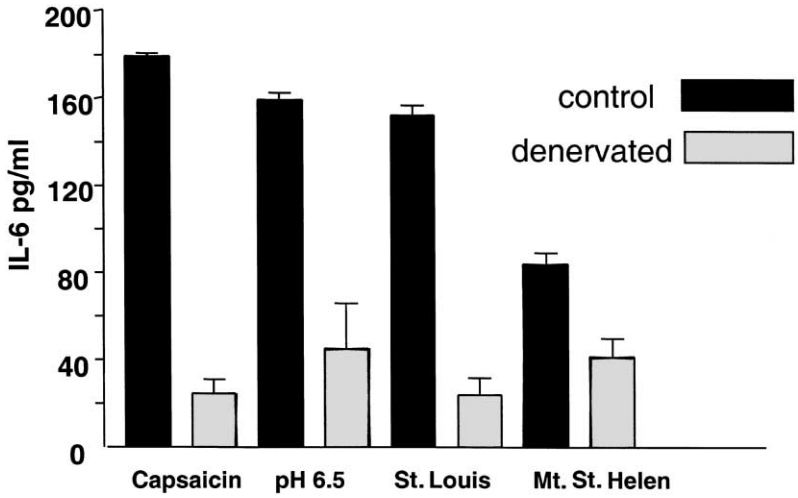

Fig. 4. BALB/c mice were denervated of polymodal sensory C fibers by neonatal capsaicin treatment. Sensory neurons, dissected from the DRG ganglia of these denervated animals and exposed to various PM (50 mg/ml) or prototype irritants failed to release IL-6 in response, implicating the sensory C fibers as critical to cytokine release in response to PM.

individual components of PM with inflammatory changes. The next series of experiments used charged, plastic synthetic particles to examine what physicochemical properties of ROFA were most responsible for activating the irritant-sensitive pathways. Principles of surface chemistry (Hunter, 1981) indicate that colloidal particles (like ROFA and other PM) carry an inherently negative surface charge (i.e. zeta potential) which attracts protons from their fluid milieu. These protons surround the individual particles, neutralizing their negative surface charge and forming a positive (i.e. acid) cloud around the individual particle (i.e. surface of shear) that has a physical dimension and a distinct electrical charge that relates to its original electronegativity (Hunter, 1981).

Since irritant receptors were purported to be sensitive to acidity (i.e. protonic charge), experiments were designed to examine if the surface charge of ROFA could stimulate inflammation. For this, the size of ROFA ''field'' (i.e. emission source) particles was measured microscopically (1–10 μm). Next, the velocity of the suspended ROFA particles was measured by microelectrophoresis and from these data, the zeta potential was calculated using the Helmholtz–Smoluchowski formula (Sennet and Olivier, 1965). By these measures, ROFA particles were shown to carry a negative surface charge, with an average zeta potential of -28 ± 1.3 mV. This value indicated that the proton concentration surrounding ROFA particles would be higher in its microenvironment relative to its surrounding solution. Thus, ROFA particles would carry a protonic micro-environment, even when suspended in a neutral buffer. We proposed that this acidic micro-environment would be sufficient to activate acid-sensitive receptors (i.e. VR1) and pathways in the airways. To examine this, 2–6 μm diameter synthetic polymer microspheres (i.e. polymethacrylic acid nitrophenylacrylate microspheres, SPM) were prepared (Eichenbaum et al., 1998) and electronegatively charged (-29 ± 0.9 mV). mV). These SPM synthetics acted as surrogates of ROFA particles with respect to their size and surface charge, but lacked other contaminants that were thought to be responsible for its toxicity (e.g. transition metals, sulfates, volatile organics and biologicals). The effects of SPM on biological activation (i.e. increases in $[Ca^{2+}]_i$, IL-6 release) were examined in tracheal–bronchial epithelial cells (i.e. BEAS-2B) and mouse dorsal root ganglia (DRG) sensory neurons that innervate the tracheal airways. These sensory neurons are known to contain abundant neuropeptide and irritant receptors, similar to those found on their fiber extensions (Baccaglini and Hogan, 1983; Gold et al., 1996; Kress et al., 1997). When these receptors are activated by irritants, they respond with a rapid increase in $[Ca^{2+}]_i$, and the subsequent release of inflammatory cytokines (i.e. IL-6) and neuropeptides from their cell bodies (Murphy et al., 1995; Marz et al., 1998).

Sensory neurons and BEAS-2B were exposed to equal numbers of ROFA or SPM particles. Biological activation (i.e. increase in $[Ca^{2+}]_i$, IL-6 release) occurred in both cell types in response to either ROFA or SPM and both responses could be reduced by either antagonist to acid-sensitive pathways (Fig. 5). Final experiments showed that neutrally charged SPM (i.e. zeta potential of 0 mV) did not produce increases in $[Ca^{2+}]_i$ or IL-6 release (Oortgiesen et al., 2000).

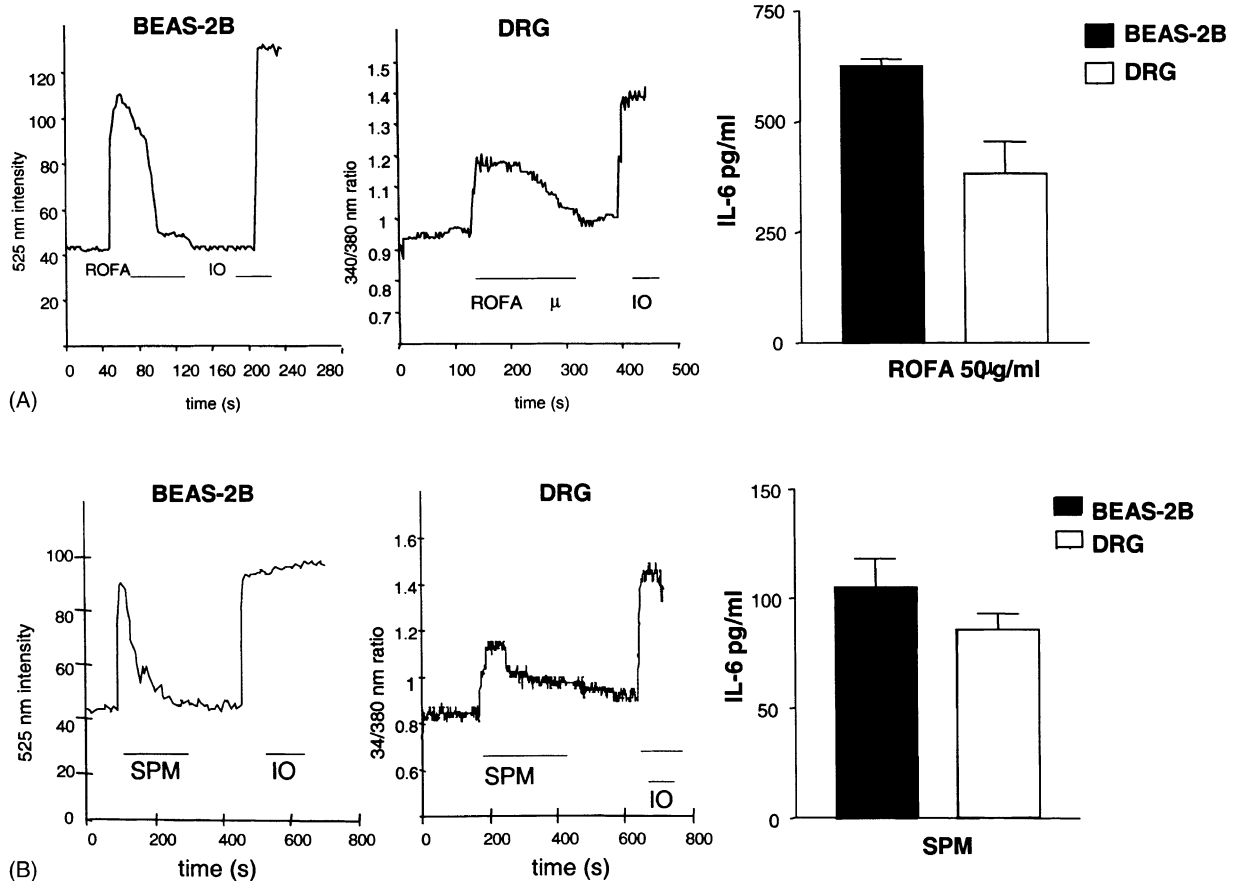

Fig. 5. DRG and BEAS-2B cells responded to (A) ROFA (50 mg/ml) or (B) SPM (2×10^4 particles/ml) with similar increases in $[Ca^{2+}]_i$ and cytokine release. Reproduced with permission (Oortgiesen et al., 2000).

Physicochemical Aspects of PM Inflammation

To expand on this observation, a larger set of PM was obtained (Table 1) from various urban (St. Louis, Ottawa), residential (Woodstove), volcanic (Mt. St. Helen), and industrial (oil fly ash, coal fly ash) sources. Morphometric analysis of microscopically visible ($\geq$2 μm) particles, suspended in buffer, indicated that each "field" (i.e. primary source) PM contained different numbers and sizes of particles. Each "field" PM was then separated into particulate and soluble fractions and described physicochemically (i.e. size and number of visible particles, acidity, zeta potential). BEAS-2B epithelial cells were next exposed to each fraction of the various PM and their biological effects (i.e. increases in $[Ca^{2+}]_i$, IL-6 release) were measured and related to their physicochemical descriptions. When examined by linear regression analysis, the only physicochemical property that correlated with increases in $[Ca^{2+}]_i$ and IL-6 release was the surface charge (i.e. zeta potential) of the various particulate fractions ($r^2 \geq 0.97$) (Veronesi et al., in press).

Hypothesis

Based on above studies, we hypothesized that the acidic micro-environments associated with negatively-charged colloidal PM particles, activated acid-sensitive receptors and pathways located on airway target cells. This neurogenic activation resulted in an immediate influx of calcium, which in turn, caused both the release of neuropeptides from sensory terminals and a more protracted release of inflammatory cytokines from sensory neurons and other airway target (e.g. epithelial) cells. Neuropeptides and cytokines then proceeded to initiate and sustain inflammatory events in the airways through the phenomenon of NGI.

SUSCEPTIBLE POPULATIONS

VR1 Irritant Receptors Linked to PM Inflammatory Response

Epidemiological studies suggest that susceptibility to PM air pollutants is variable among sensitive

populations (i.e. the young, the elderly, those with pre-existing respiratory and cardiopulmonary conditions). Demographics, life-style (e.g. smoking), and pre-existing pathologies have been proposed to explain this variability (Dockery and Pope, 1994; Pope, 2000; Utell and Frampton, 2000). Animal studies of pollutant-induced airway inflammation are also marked by species and strain differences. Genetic differences are thought to underlie these variations and have been experimentally demonstrated for ozone (Kleeberger, 1995; Zhang et al., 1995), nitrogen dioxide (Holroyd et al., 1997) and diesel exhaust (Ichinose et al., 1997; Miyabara et al., 1998). Several studies also indicate that the airway inflammation and hyper-responsiveness that occur with ROFA exposure are strain- and species-specific (Gavett et al., 1998; Kodavanti et al., 1997b).

To investigate the underlying causes of this selective susceptibility, we exposed BALB/c and C57blk/6 (i.e. B6) mice intratracheally to ROFA and examined them for signs of airway inflammation. Bronchial lavage of BALB/c mice showed significantly higher numbers of neutrophils and increased airway hyper-responsiveness in response to methacholine challenge, whereas B6 mice showed no significant change in either inflammatory endpoint. To examine the mechanism of this strain specificity to PM, cultures of DRG sensory neurons, which innervate the upper airways in situ, were explanted from both BALB/c and B6 fetal mice and exposed to ROFA, woodstove dust, ambient PM from Ottawa and St. Louis, coal fly ash and oil fly ash or to prototype irritants (e.g. capsaicin, acid pH). These data showed that DRG neurons from BALB/c mice consistently released significantly higher levels of the pro-inflammatory cytokine IL-6 into their nutrient media relative to neurons from B6 mice (Fig. 6). DRG neurons cultured from BALB/c and B6 neonates, were also examined by calcium imaging for changes in response to acid pH (5.0, 6.5) or to the botanical toxin, capsaicin. These data showed that significantly higher numbers of BALB/c neurons responded to these prototype irritants with increases in $[Ca^{2+}]_i$, relative to B6 neurons. Morphometric analysis of BALB/c neurons, histochemically stained with cobalt to label neurons bearing capsaicin-sensitive (i.e. VR1) receptors, showed a significantly higher level of stained neurons relative to B6 neurons. Finally, semi-quantitative RT-PCR showed a higher expression of VR1 receptor mRNA in DRG taken from neonatal BALB/c mice relative to B6 mice (Veronesi et al., 2000). Subsequent experiments, using similar techniques (i.e. RT-PCR, cobalt histochemistry and immunocytochemistry), demonstrated a higher expression of SP (NK1) neuropeptide receptors and release of neuropeptides in

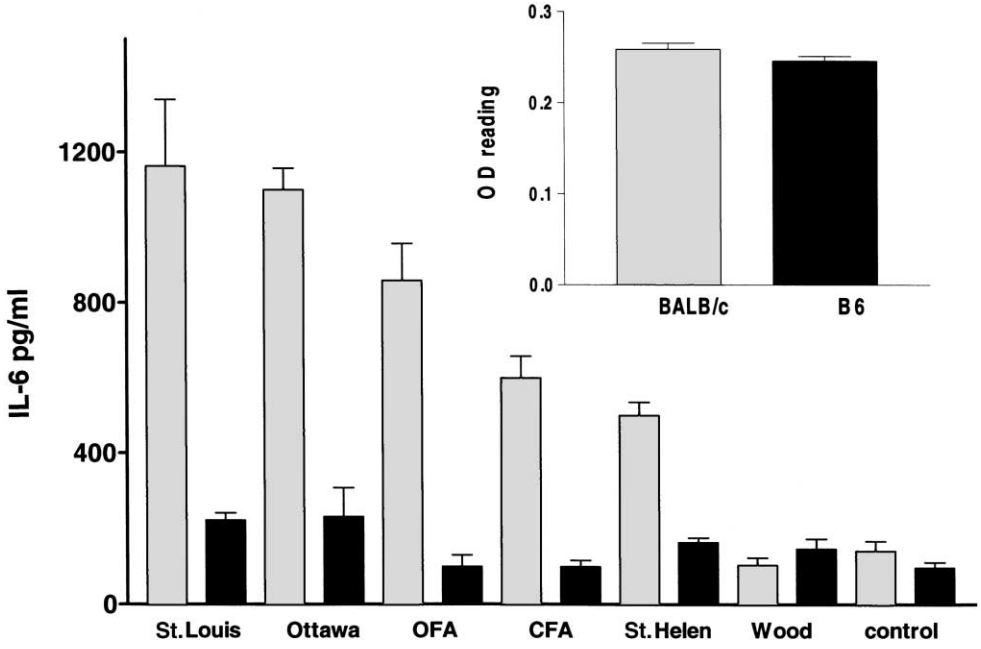

Fig. 6. DRG neurons, dissociated from fetal BALB/c and B6 mice were exposed to various PM (50 μg/ml) for 4 h. In all instances, except for woodstove, BALB/c released significantly higher levels of IL-6 relative to B6 ($P < 0.05$). A neutral red viability/cytotoxicity (inset) showed no differences in BALB/c and B6 control cultures at the time of PM treatment. DRG were isolated, cultured at identical cell densities (2×10^5 cell/ml), and exposed under similar conditions. OFA: oil fly ash, CFA: coal fly ash, MSH: Mt. St. Helen. Reproduced with permission (Veronesi et al., 2000).

response to PM in sensory neurons taken from BALB/c mice relative to the B6 strain.

Taken together, the above in vivo and in vitro studies suggested that the variable inflammatory sensitivity to PM observed in different mouse strains (i.e. BALB/c, B6) related to quantitative differences in the neuropeptide, VR1 receptors and acid-sensitive pathways found on sensory neurons that innervate the nasal and upper pulmonary airway. Such data showed how genetically-determined differences in sensory neural pathways could influence expressions of PM-induced airway inflammation. Current experiments are directed at understanding how non-genetic factors can alter PM sensitivity through neurogenic pathways.

Non-Genetic Factors that Affect VR1 Receptor Sensitivity

Cell–Cell Interactions: Neuron–Glia Relationships

Neuron–glial interactions are reported to influence receptor sensitivity and cell sensitivity to chemical toxicants (McMillian et al., 1995; Brismar, 1995; Belmonte et al., 1996). DRG somatosensory neurons and their peripheral extensions are surrounded by several types of glia (e.g. satellite cells, astrocytes, Schwann cells) which contain neuropeptide and capsaicin receptors (Brismar, 1995; Palma et al., 1997). Preliminary data indicate that cultured glial cells found in the DRG's environment respond to PM, acid pH and capsaicin with increases in $[Ca^{2+}]_i$ and inflammatory cytokine release and that the reduction of this glial environment with anti-mitotic treatments (e.g. arabinoside) significantly enhances IL-6 release in DRG sensory neurons exposed to PM (Fig. 7). Such data suggest that disruption of the sensory neuron–glia integrity in situ can alter (i.e. enhance) the receptor-mediated sensitivity to PM, an observation that carries clinical significance since various metal toxicants (e.g. methyl mercury, tellurium) and pharmaceuticals (e.g. taxol, cisplatin) are known to damage the peripheral and central glial population (Thomas et al., 1988; Hoi et al., 1994; Aschner, 1998).

Cell–Cell Interactions: Neuron–Epithelial Relationships

In normal physiological settings, the respiratory epithelial population and its sensory innervation act reciprocally to influence the growth, differentiation, and homeostasis of each other (Chan and Haschke, 1985; Garcia-Hirschfeld et al., 1994; White et al., 1995; Belmonte et al., 1996). These relationships are especially critical to the organism's inflammatory response (Hoyle et al., 1998; Ogun-Muyiwa et al., 1999; Shu and Mendell, 1999). The epithelial brush border which line the tracheal–bronchial airways contain neutral endopeptidase (NEP), an enzyme which acts to deactivate inflammatory neuropeptides (e.g. substance P) released by the sensory nerve fibers (Devillier et al., 1988; Piedimonte et al., 1992; Di Maria et al., 1998). Since the epithelial cells, and their

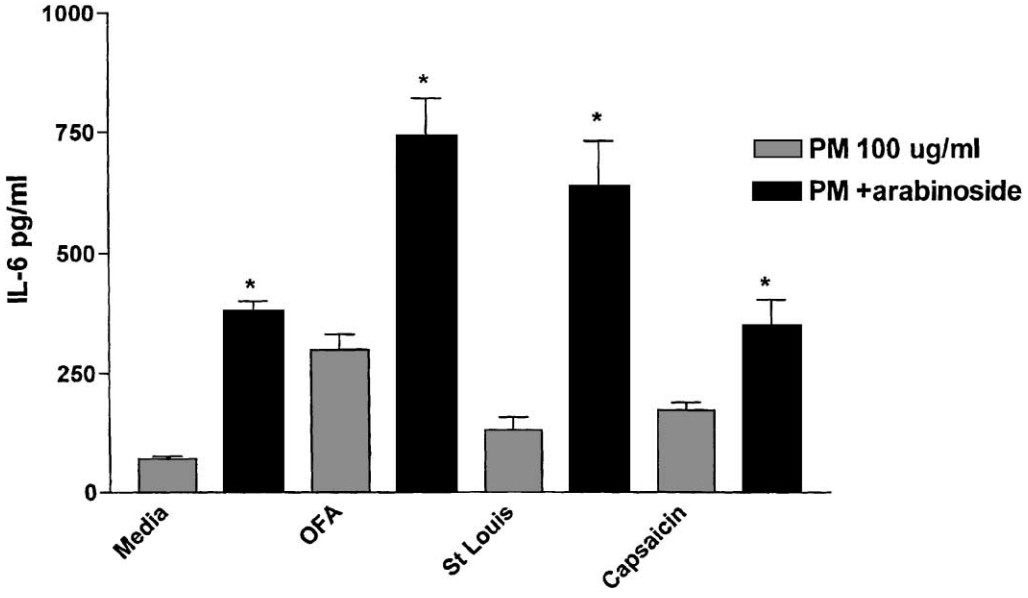

Fig. 7. DRG were cultured in the presence or absence of their glial environment by cytosine arabinoside (6 μM) treatment for 48 h. Both cultures were then exposed for 4 h to various PM (100 mg/ml). DRG were isolated and cultured at the same cell densities and exposed under similar conditions. Significance ($^*P < 0.05$) is indicated.

sensory C fibers terminals are the initial targets of inhaled PM (Ohtoshi et al., 1994; Mills et al., 1999), studies were designed to examine their relative biological response to PM. BEAS-2B epithelial cells and DRG neurons were exposed at equivalent concentrations and exposure times to PM from industrial, ambient, residential, and volcanic sources. In all instances, sensory neurons released 10–200-fold higher levels of IL-6 relative to epithelial cells (Veronesi et al., in press). This disproportionate inflammatory response of two critical airway target cells may be relevant to the susceptible population component of PM inflammation since many examples of respiratory disorders (e.g. asthma, chronic allergen exposure, viral respiratory infections (Lazarus, 1986; Wasserman, 1988; Tonnel et al., 1992; Elwood et al., 1993; Ladenius et al., 1995; Widdicombe, 1996b; Folkerts and Nijkamp, 1998; Polito and Proud, 1998) and conditions associated with chemical pollutants (e.g. cigarette smoke toluene diisocyanate, ozone) (Gordon et al., 1985; Laitinen et al., 1985; Tonnel et al., 1992; Khair et al., 1996; Baeza-Squiban et al., 1999) are characterized by damage to the epithelial barrier that lines the airways. Such damage, not only results in the loss of critical neuropeptide deactivating enzymes (e.g. NEP), but allows the sensory fiber to physically extend closer to the airway lumen and in closer proximity to the inhaled PM particles. These data (Veronesi et al., in press) which show a higher inflammatory sensitivity of sensory neurons relative to the epithelial cell population, suggest that disruption of the epithelial barrier lining the lumen would result in enhanced and prolonged inflammatory events. Although ambient levels of particulates, vapors, and aerosol pollutants may have little consequence to healthy individuals whose lungs have intact epithelial linings, those sensitive individuals whose epithelial lining is damaged (e.g. smokers, asthmatics, elderly) would experience an increased inflammatory response to PM and other pollutants.

SUMMARY

The studies described in the present review support a neurogenic explanation for the inflammation associated with PM air pollution collected from multiple sources. Our data report that airway cells initially targeted by the inhaled PM contain acid-sensitive irritant receptors whose activation by the surface charge carried on PM particles results in cellular inflammation. A neuroimmunological mechanism in the initiation of PM-associated airway inflammation is plausible since sensory receptors are highly sensitive indicators of noxious and potentially damaging chemical exposure. This neurogenic hypothesis has been used to examine the sensitive sub-population component of PM and current research extends this dataset by examining how non-genetic factors (i.e. cell–cell interactions) modifies receptor sensitivity. Such studies promise to offer insight into predisposing or acquired conditions that may contribute to the sensitive subpopulation phenomenon of PM exposure. This neuroimmunological explanation for PM inflammation, while novel, is firmly supported by neurobiological, immunological, and pulmonary interactions which culminate in the pathophysiology of neurogenic inflammation.

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