opposed to cell surface receptors is that only a subset of the total receptor response is affected. Mitogenic signallings appear to conform to an hourglass model (see figure). Pathways converge onto conserved sequentially acting protein kinases and diverge again further downstream at the level of the MAPK superfamily. These kinases have a broad range of different substrates. Thus, further refinement of the approach may be achieved by focusing on more downstream elements of specific signalling cascades.

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Nitric oxide in mucosal immunity

Nitric oxide induced by γδ T cells (pages 552–557) and derived from dietary nitrate (pages 546–551) may limit microorganism growth.

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Nitric oxide is produced by many different animals, ranging from mammals to insects. Besides animals, an assortment of organic compounds (for example, nitroglycerin) and spontaneous chemical reactions (for example, acidified nitrite) generate nitric oxide. In this edition of *Nature Medicine*, two reports describe how endogenous (synthesized by mammalian cells) and exogenous (chemically generated) sources of nitric oxide serve as a physiological cytoprotective for the mucosa[1,2].

Despite its small size and short life span, nitric oxide is involved in a number of diverse and complex biological processes[3]. The ability to affect these processes is partially attributed to the physical and chemical make-up of nitric oxide. As a gaseous free radical, nitric oxide contains an extra electron, thus making it highly reactive with a variety of different molecular targets, including iron-responsive proteins (such as guanylate cyclase and the cytochrome P-450 enzymes). The interaction between nitric oxide and such targets in the body can lead to a functional response[4].

The magnitude and duration of nitric oxide synthesis by a mammalian cell determines whether its actions are regulatory or cytotoxic. Pulses of low amounts of nitric oxide are involved with regulatory or housekeeping events, such as maintaining vascular tone. Large amounts of nitric oxide, produced for extended periods of time, are often associated with a disease state. Such high levels of nitric oxide are cytotoxic and are controlled immunologically[5].

Viruses, mycobacteria, protozoans, helminths, bacteria and fungi (such as *Candida albicans*) are among a growing list of microbes that are susceptible to nitric oxide. The mechanism(s) by which nitric oxide exerts antimicrobial activity is not clearly identified. Attack by nitric oxide and reactive nitrogen intermediates (such as peroxynitrite) on vital iron-dependent enzymes has been thought to contribute to metabolic dysfunction[6,7]. A decrease in cellular respiration and replication has been shown to be associated with high levels of nitric oxide produced by cytokine-activated macrophage cells[1].

Jessica Jones-Carson, Edward Balish and collaborators report that γδ T lymphocytes are involved in host resistance to orogastric candidiasis, in part through the induction of nitric oxide synthesis[1]. They found that depletion of γδ T cells blocked expression of nitric oxide synthesis and dramatically increased susceptibility of mice to mucosal candidiasis in the mouth and stomach. Along similar lines, others have hypothesized that nitric oxide in the stomach may control gastric ulceration by inhibiting the growth of *Helicobacter pylori*[8].

A unique property of γδ T cells is that they recognize antigens directly, without the need for specialized antigen-presenting cells[10]. In response to antigenic stimulation, they are able to secrete cytokines, such as interferon-γ (IFN-γ). Jones-Carson *et al.* provide convincing evidence that the IFN-γ produced by *C. albicans*-elicited γδ T cells stimulate macrophage candidacidal activity in culture[1]. However, it remains unclear whether macrophages are the principal source of nitric oxide in candida-infected mice. Instead, epithelial cells lining the orogastric tract might be stimulated by neighboring γδ T cells to produce nitric oxide. Non-macrophage cells have been shown to express nitric oxide-mediated cytotoxicity, including epithelial autotoxicity to pertussis[10], endothelial destruction of tumour[11] and *Schistosoma mansoni*[12], fibroblast injury of *Chlamydia trachomatis*[13] and hepatocyte suppression of malarial sporozoite growth[14,15].

Because of the uncertainty of whether human macrophage cells generate nitric oxide, the relevance of studying nitric oxide in mouse macrophages to understand human disease is frequently called into question. Human synthesis of nitric oxide synthesis is established; the expression of nitric oxide has been reported in a number of human cells, including respiratory epithelium, hepatocytes and macrophages. Unfortunately, there are as many negative reports as there are (unconfirmed) positive reports on nitric oxide synthesis by human macrophages.

Nevertheless, nitric oxide is a potent antimicrobial agent. The experiments of Callum Duncan and co-workers in Nigel Benjamin's lab identified a non-immunological source of nitric oxide:

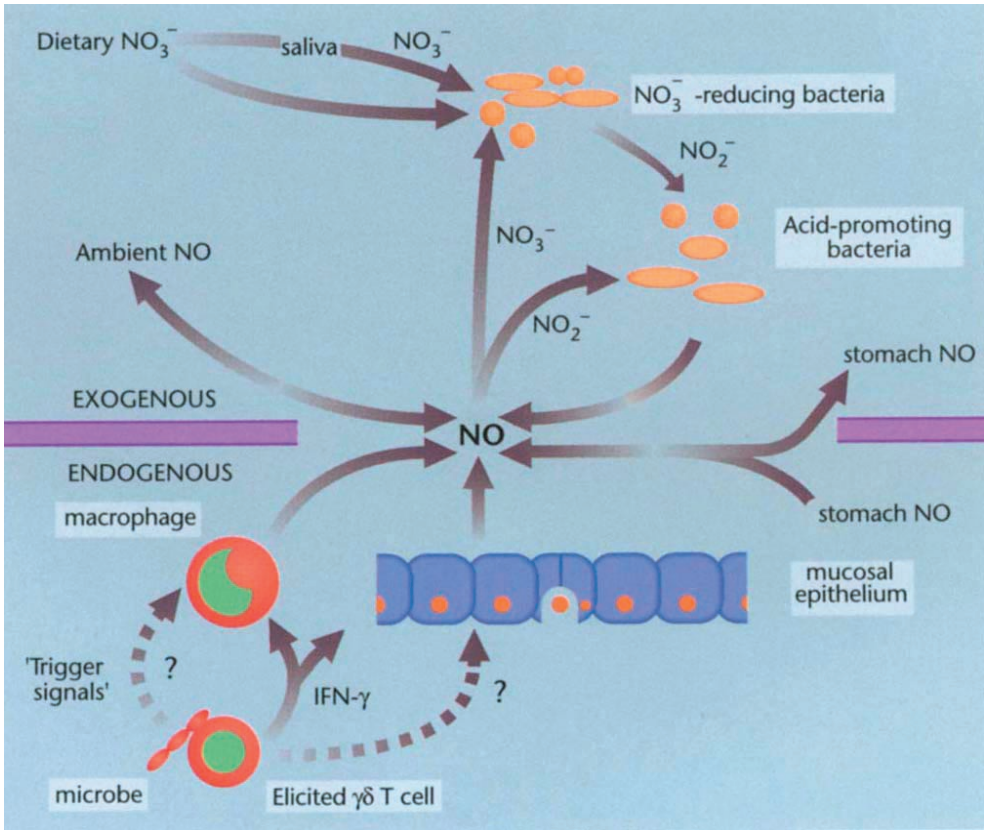
immunological source of nitric oxide: dietary nitrate may contribute to the host defences in the oral cavity[2].

The thought of ingesting foods rich in nitrate to promote oral hygiene is somewhat ironic. A number of articles over the past 30 years have reported on the carcinogenic activity of nitrate participating in the endogenous formation of cancer-promoting nitrosamines[1-7]. Ingestion of amines and nitrates has been associated with tumour formation in animal models. Such reports have caused health-conscious individuals to shy away from cured meats and foods preserved with nitrate and nitrite. More recent epidemiological studies, however, have failed to demonstrate an association between nutritional nitrate and gastric cancer.

Clearly, Duncan *et al.* do not dismiss the harmful and potentially carcinogenic activity of nitrosamines, but instead consider the beneficial effects of nitrate: Nitric oxide chemically generated from nitrate in the mouth may keep oral microbes in check. Reminiscent of earlier studies showing that nitrate reduction to nitrite is anatomically restricted to the tongue surface, Duncan *et al.* demonstrate that this process is enzymatically driven by the bacterial microflora. Interestingly, they found nitrate reduction to be limited exclusively to the posterior surface of the tongue. This area is inhabited by Gram-positive and Gram-negative bacteria, both a likely source of nitrate reductase. Upon further reduction or acidification of nitrite, nitric oxide is generated. In sharp contrast, tongues from animals in a germ-free environment showed no nitrite, which suggests that nitrate reduction is a bacterial rather then a mammalian process[2].

The authors present the reasonable hypothesis that the conversion of nitrite to nitric oxide may be due to the acid-producing bacteria residing in the gingival sulcus surrounding teeth. If so, the gingiva will be exposed to significant amounts of nitric oxide, because the acidic pH within the sulcus will continuously regenerate nitric oxide from nitrite. It would not be surprising if gingivitis is a consequence of long-term exposure to high levels of nitric oxide. In this instance, limiting dietary nitrate intake may be more beneficial. As to whether excess amounts of nitric oxide are produced in heavily colonized periodontal pockets and whether this contributes to tissue destruction remain to be seen; however, removal of acid-producing bacteria from the gingival sulcus and margin is often the first step in the treatment of gingivitis and periodontal disease.

Does nitric oxide limit the growth of the very microbes that produce these oxides of nitrogen? An intriguing thought expressed by the investigators is that the nitrate-reducing bacteria have adapted by restricting their growth to an area on the tongue that is relatively alkaline, thereby shielding themselves from acidified nitrite and subsequent nitric oxide-mediated injury[1]. Alternatively, bacteria in the oral



Multiple sources of nitric oxide (NO) in the oral cavity. Exogenous sources of NO: inhalation of ambient air, diffusion from stomach and lung and conversion of nitrate (NO_3^-) by facultative anaerobic bacteria to NO. Concentrated in and secreted by salivary glands, NO_3^- is reduced to nitrite (NO_2^-) by the bacterial microflora that colonize the posterior portion of the tongue. NO is subsequently generated from NO_2^- by acidification; following a carbohydrate meal, bacteria located at the gingival margin reduce plaque pH sufficiently to convert NO_2^- to NO. Excess NO undergoes further oxidation (NO_3^-) and NO is then regenerated via reduction and acidification. Endogenous sources of NO are derived from the guanidino nitrogen of L-arginine, a reaction that is catalysed by the enzyme NO synthase, which exists in several isoforms. Expression of the inducible isoform occurs in a variety of cells (including macrophages and some types of epithelial cells) and is characterized by the release of large, cytotoxic amounts of NO. Elicited-intraepithelial γδ T cells can stimulate the expression of inducible-NO synthase via IFN-γ. At present it is unclear whether the mucosal epithelium lining the orogastric tract is the source of NO, although stimulated macrophages can kill pathogens by production of NO. Other proinflammatory cytokines, such as tumour necrosis factor and interleukin 1β, and microbial products may serve as a 'trigger signal' to enhance NO release and associated antimicrobial activity (dotted line).

oxide by upregulating the expression of genes, triggered by nitric oxide and superoxide, that confer resistance to these oxidants[18,19].

In consideration of these two reports, it would be interesting to know whether dietary nitrate decreases oral thrush on the tongues of *C. albicans*-infected mice depleted of γδ T cells. Such observations may eventually become relevant in the managed care of individuals who are malnourished or immunocompromised with complicating infections, such as orogastric and mucocutaneous candidiasis. A multivalent treatment strategy consisting of antifungal drugs administrated with both nitric oxide-inducing immunomodulators and dietary nitrate supplements may someday be a mainstay. At the very least, the authors heighten our awareness of the potential consequences of disrupting the nitric oxide-promoting microflora. They also provide a compelling argument for the need to revisit the role of dietary nitrate as a component of the host's defensive armament.

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Measuring NO_2 exposure in respiratory disease

Pollutant research is moving indoors as scientists uncover new links between indoor exposure and disease. However, measuring the levels of exposure is a difficult task.

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Interest and activity in indoor air and health-related research is increasing. Researchers now know that most pollutant exposure occurs indoors, and many pollutants are generated from indoor sources. Nitrogen dioxide (NO_2) in particular, formed indoors from tobacco smoke and combustion in gas appliances without flues, is recognized as an important indoor pollutant. Many families are exposed to NO_2 in their homes, and children, who are especially susceptible, are exposed both at home and in schools that use gas heaters without flues for classroom heating during winter months. Health effects known to be related to NO_2 exposure include increased respiratory symptom production[1], impaired lung function[2] and increased bronchial reactivity[3], especially in people suffering from asthma.

However, epidemiological studies of the association between NO_2 exposure and respiratory illness have yielded mixed results[1,4-6]. The lack of consistent findings may in part be due to the absence of objective measurements of NO_2 in some studies, and the use of retrospective questionnaires to obtain illness histories, with the former thought to result in misclassification of subjects for analysis, and the latter likely to involve recall bias[7]. Another reason may be the absence of specific mixtures of pollutants indoors, such as NO_2 and SO_2, which together have been shown to enhance bronchial reactivity to allergens in mild asthmatics[8]. Inconsistent findings may also be related to the way that NO_2 has been monitored for use as a measure of NO_2 exposure in past studies. The preferred approach has been the measurement of average levels of NO_2 over one to two weeks. This method does not take into account the daily short-term (for example, one hour) peak levels that can be four or more times the usual background levels. This method needs to be critically examined.

In a recent *Lancet* article, W. Tunnicliffe and co-workers[9] reported on their study of ten non-smoking asthmatic subjects, each of whom were exposed for one hour to either air, 100 parts per billion (ppb) NO_2 or 400 ppb NO_2 in double-blind random order. Immediately following exposure to either air or NO_2, each subject underwent a fixed-dose challenge with house-dust mites, a known potential asthma precipitant. Significant adverse effects were demonstrated at 400 ppb. The findings suggest that at concentrations often experienced in the home as daily peak levels, NO_2 can enhance asthmatic responses to allergen from house-dust mites.

Advantages of experimental studies such as this include the ability to selectively recruit specific types of individuals; knowledge of the nature, duration and level of exposure; and the opportunity to study pulmonary responses in detail[10]. The major disadvantage is that subjects are exposed for only a short time under experimental conditions that do not necessarily reflect outcomes that would be obtained in normal environments. Nevertheless, the results of the above and other experimental studies[11,12] suggest that short-term exposure to NO_2, at levels often experienced as daily peak levels, is associated with adverse respiratory effects.

In an attempt to link NO_2 to a specific biological effect, M. Frampton and colleagues investigated the effects of NO_2 inhalation *in vivo* on the ability of human