expect, mice deficient of IL-6, so called IL-6 knockout mice, have impaired immune responses[12]. Although the current study did not demonstrate any significant consequences of the receptor antagonist immunizations in mice, the formation of anticytokine–cytokine immune complexes in humans could result in significant pathology related to kidney, joint, and vascular disease. Heterogeneity of human immune responses may also be problematic for receptor antagonist immunizations; some human individuals may simply not mount a significant humoral response.

Another limitation of any cytokine neutralization strategy relates to the existence of more than one cytokine with pathophysiologic consequences in a given disease. In multiple myeloma, a common lymphoid malignancy of antibody-producing plasma cells, hIL-6 induces proliferation and inhibits apoptosis of the malignant cells[8]. Recently, work in our laboratory has suggested that the Kaposi's sarcoma-associated herpesvirus (KSHV) plays a causal role in myeloma by expressing a homolog to hIL-6, termed viral IL-6 (ref. 13). This viral IL-6 retains the biological activity of its human counterpart, yet can be immunologically differentiated from hIL-6 (ref. 14). Consequently, anti-hIL-6 antibodies induced by immunization with hIL-6 receptor antagonists may not recognize the viral IL-6. Cross-reactivity of the anti-hIL-6 antibodies with viral IL-6 is relevant not only for myeloma, but for other neoplasms (i.e., Kaposi's sarcoma, pleural effusion lymphoma, and multicentric Castleman's disease) associated with both hIL-6 and viral IL-6.

Immunization with receptor antagonists promises to provide a novel, exciting, and effective strategy for neutralizing cytokines and other ligands that are involved in the pathogenesis of disease. The potential applications to inflammatory, autoimmune, malignant, and degenerative diseases is virtually endless. The development of neutralizing antibodies to the therapeutic agent, one of the potential limitations of monoclonal antibody strategies, is the basis for the success of receptor antagonist immunization. Thus, a problem becomes a solution.

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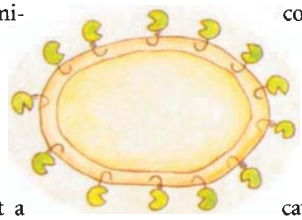
Turning poison eaters inside out

Simon Silver and Amit Gupta

It is obviously better to detoxify nasty chemical compounds on the outside of the cell, rather than to take them up in order to be digested by intracellular enzymes. In this issue, Wilfred Chen and colleagues[1] have done just that, expressing organophosphate hydrolase on the surface of a strain of *Escherichia coli*, resulting in an organism that can inactivate a group of toxic chemicals, ranging from organophosphorous pesticides, such as parathion and paraoxon, to the chemical warfare agents sarin and soman. Toxin removal using this recombinant organism could represent a more effective and cheaper alternative to current landfill and incineration methods.



Organophosphate hydrolase cleaves P–O, P–F, and P–CN bonds found in a wide range of pesticides and chemical toxins, has no requirement for intracellular cofactors, and is found in a variety of soil microbes. Attempts have been made to immobilize the purified enzyme on to a variety of surfaces in order to assess its potential in bioremediation. However, the cost of purifying the enzyme and making the reactor has proved prohibitive. Immobilized natural bacteria can also be used, but mass transport limits the movement of substrates and products across cell membranes, resulting in a process that is too slow and ineffective.

In their paper, Richins et al.[1] have isolated the organophosphate hydrolase gene from a soil microbe and joined it to a gene fusion construct[2] encoding the signal sequence, the first nine amino acids of lipoprotein (Lpp), and the first 114 amino acids of a major *E. coli* outer membrane protein (OmpA). Lpp anchors the protein to the outer surface of the bacterium. OmpA passes across the outer membrane five times, providing a junction point on the surface of the cell for the organophosphate hydrolase enzyme. Overall, the anchored enzyme faces outwards, away from the cell and "toward" the pollutant; it is not trapped in the periplasm. This system allows maximum overexpression and anchoring of the fusion protein to the cell surface where it can be of best use.

Such genetic constructs are themselves often toxic to the cell and therefore must be under careful regulation so that the surface-arrayed enzyme is only made on specific demand. That is the case here. In trial experiments described in their paper, the enzyme is overexpressed and 10-fold more accessible to substrate than when made as an intracellular enzyme. Current efforts testing these cells immobilized and in pollutant-containing bioreactors are now underway.

With luck and effort, this type of genetically engineered bacterium will be useful for bioremediation of polluted waters and soils. Water detoxification is easier as the substrate can pass through columns containing immobilized recombinant cells. Soil bioremediation may, however, present a more thorny challenge.

First, the bioavailability of the pollutant to the bacterial surface enzyme is a serious problem. If the pesticides and warfare agents are firmly fixed to soil particles, then they may not be accessible to the bacterial enzyme. This can be an insurmountable limitation on bioremediation, even when chelates, surfactants, and other agents are used to liberate substrates. Second, the release of genetically modified organisms in the field still raises issues of social and/or political acceptability. The current near-global trend in which recombinant microorganisms are percieved poorly by the public presents another barrier to the application of this technology in the field (rather than the more limited use with pumped liquids).

In the meantime, one hopes that recombinant bacteria, such as Chen and colleagues' strain, will be candidates for limited release, especially following the current US Department of Energy (Germantown, MD) field trial with polynuclear aromatic hydrocarbon degrading bacteria at the site in Oak Ridge, Tennessee.

Currently, we are caught between Scylla and Charybdis: Leave pesticides and warfare chemicals in the soil or carry out costly and harsh physical detoxification methods (e.g. incineration). Concerted efforts focused on moving effective recombinant bioremediation organisms out of the laboratory and into the field could provide another answer.

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