A transformed view of cyclosporine

Gary J. Nabel

Many transplant patients are given the drug cyclosporine to suppress their immune systems and prevent rejection. But cyclosporine also increases the risk of cancer, always thought to be a side effect of the depressed immune system. A new study shows that cyclosporine directly affects tumour growth and may be the culprit.

Cyclosporine and its relative FK506 are drugs used to treat autoimmune diseases and prevent rejection in bone marrow and organ transplantation. Originally identified by screening for antibiotics from microorganisms, these remarkable drugs suppress the immune system by altering activation of the genes that encode immune factors. However, this suppression must be balanced with the need to maintain enough strength in the immune system to combat infection. So, side effects of cyclosporine treatment are not uncommon, and include an increased risk of cancer in patients who take these drugs long-term.

On page 530 of this issue, Hojo *et al.*[1] describe how they have re-examined the presumed cause of cyclosporine-associated cancers. It was previously held that such cancers result from a failure of the immune system to eliminate cancerous cells — presumably because, when treated with cyclosporine, the immune system cannot detect and respond to proteins associated specifically with the tumours. But Hojo and colleagues suggest a radically different explanation. They find that cyclosporine itself alters the characteristics of several cancerous cell lines *in vitro* and *in vivo*. The authors believe that it does this by inducing the synthesis of another molecule known as transforming growth factor-β (TGF-β).

Hojo *et al.* first found that, when exposed to cyclosporine *in vitro*, cancerous cells become more likely to divide, move and spread — they change shape, show increased mobility and, unlike normal cells, can grow without being anchored to a solid surface. Moreover, when the authors injected different types of tumour cells into immune-deficient mice, more secondary tumours developed in the lungs in the presence than in the absence of cyclosporine. These results challenge assumptions about how cyclosporine-associated tumours arise, and how immune surveillance is involved in the development of cancer. They also raise questions about how cyclosporine suppresses immune function, suggesting a more general role than previously thought for cyclosporine-dependent signalling pathways in human disease.

The mechanism of cyclosporine action has been described by several laboratories (reviewed in refs 2, 3). It was thought to block the immune system by inhibiting signalling through the T-cell receptor. The effect of this is to prevent the production of cytokines that would normally stimulate an immune response (Fig. 1). But this explanation is not necessarily complete. First, when one of these cytokines, interleukin-2, is disrupted in transgenic animals, the effect on immune function is not the same as treatment with cyclosporine[4–7]. Second, if another molecule in the proposed signalling pathway (the nuclear factor of activated T-cells, NF-AT) is knocked out, again the effects do not match treatment with cyclosporine[8].

An alternative explanation comes from the fact that cyclosporine stimulates the production of TGF-β[9,10]. We do not know how it does this, but it is an important avenue of further investigation because several previously mysterious side effects of cyclosporine can be explained by the induction of TGF-β (Fig. 2). For example, cyclosporine can cause liver damage, and thickening and scarring of the kidneys and skin — an effect also seen with increased TGF-β[11]. Because TGF-β is itself a well-known and potent immunosuppressive agent, increased production of TGF-β — as wellas the inhibition of cytokine production — could explain the activity of cyclosporine.

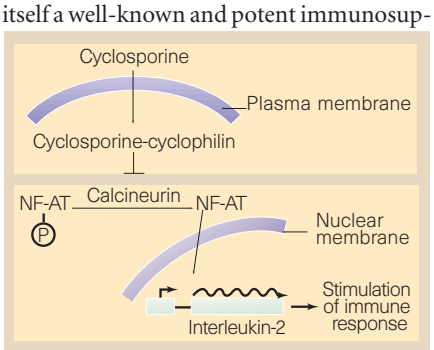

Figure 1 **Previously defined mechanism for the action of cyclosporine. After binding to cyclophilin, cyclosporine inhibits calcineurin, which normally removes a phosphate group from members of the nuclear factor of activated T-cells (NF-AT) family. This dephosphorylation normally promotes movement of NF-AT to the nucleus, where it stimulates expression of the genes for interleukin-2 and other stimulatory cytokines. Inhibition by cyclosporine blocks cytokine expression and prevents an immune response.**

Hojo *et al.*[1] have now recognized that, as well as affecting immune function, TGF-β might alter the behaviour of cancerous cells. The remarkable changes that they observed in cyclosporine-treated cancerous cell lines were reversed when they added an antibody that bound to (and therefore blocked the action of) TGF-β. In the same way, the increased spread of cancer cells *in vivo* was also blocked by the TGF-β antibody. These results indicate that treatment with cyclosporine does not increase tumour growth indirectly, by a failure of the immune system, but rather directly through a non-immune mechanism that acts on the tumour itself via TGF-β receptors.

Hojo and colleagues' study is certainly provocative, calling into question the proposed mechanism by which cyclosporine induces secondary cancers. But several caveats should be noted. For example, we do not know whether cyclosporine has a similar effect on precancerous cells, or whether it is involved in converting cells from a benign to a cancerous state. Nonetheless, the data do indicate that cyclosporine and its relatives could exacerbate tumour growth in patients with existing tumours.

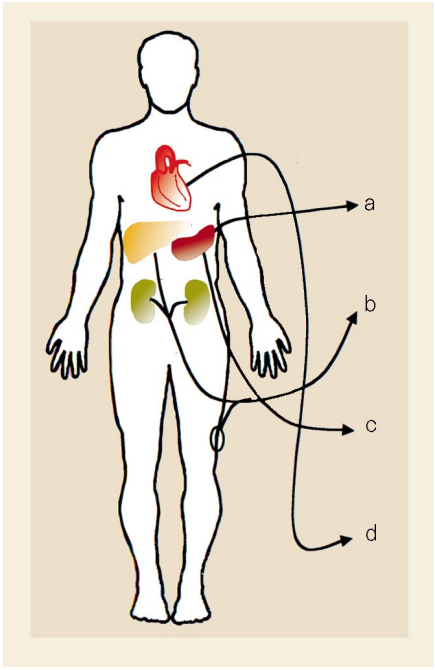

Figure 2 **Emerging ideas about the action of cyclosporine. Hojo and colleagues[1] have shown that cyclosporine may cause cancer by stimulating production of the transforming growth factor-β (TGF-β), although it is not yet known how it does this. TGF-β may then:** a, **stimulate the growth of existing cancers;** b, **cause liver damage, and thickening and scarring of the skin or kidneys; or** c, **suppress immune responses.** d, **Cyclosporine may also be used to treat heart disease by inhibiting thickening of the heart muscle.**

Other studies point to a broader involvement of the signalling pathway triggered by cyclosporine in tissues outside the immune system. Molkentin, Olson and colleagues[12], for example, have reported that increasing the expression of activated NF-AT3 in the heart muscle of transgenic mice leads to enlargement of the cardiac tissue (cardiac hypertrophy). Cardiac hypertrophy can be inhibited by giving mice cyclosporine[13], although it is not clear whether TGF-β is the mediator involved here. An alternative explanation may come from the finding that cyclosporine-mediated inhibition of calcineurin blocks the expression of muscle-specific genes, preventing hypertrophy[14]. Whatever the mechanism, these results indicate that a cyclosporine-like agent could be used to treat heart disease. However, such treatment would probably not be given unless the effects of cyclosporine on the immune system could somehow be reduced.

Hojo and colleagues' study will raise concerns about the increased incidence of cancer associated with cyclosporine treatment. This complication is, however, a well-known side effect of cyclosporine therapy, one that is currently balanced against the need to treat life-threatening diseases. The new observations do not alter this risk, nor do they suggest that any additional precautions be taken beyond those already recognized. But they do provide an insight into how these cancers come about, and may be useful in treating them. The results could also provide a lead in the search for new immunosuppressive drugs that might be more selective. But cyclosporine, whose mechanism of action was thought to be well understood, remains enigmatic in many respects. □

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Particle physics

The undemocratic proton

David J. Miller

Since the proton is the lightest stable composite object in the Universe we might expect it to be the simplest. Yet its structure is much more complicated than we can explain with current theories, as reported in *Physical Review Letters* by the HERMES collaboration[1] at the HERA accelerator in Hamburg and by the NuSea collaboration[2] at the Fermilab Tevatron near Chicago. HERMES used semi-inclusive deep-inelastic scattering of a positron beam to identify the contribution of individual quarks to the momentum of the proton. Both groups see an excess of virtual down-antiquarks over up-antiquarks, contradicting predictions that the two flavours of antiquark should be present inside the proton in equal numbers.

Elastic scattering is what happens with billiard balls — they collide and bounce without changing their identity and with no significant loss of kinetic energy. Inelastic scattering usually means that one or both of the colliding objects has been changed by the collision, in that they may be deformed or broken into pieces like a billiard ball shattered by a bullet. Deep-inelastic scattering is a subtle amalgam of the two, as if a bullet shatters a billiard ball but in the process makes a quasi-elastic collision with a heavy, compact ball-bearing buried inside the target. Particle physicists began to believe in the reality of quarks in the early 1970s (ref. 3), when deep-inelastic scattering experiments with electrons (bullets) on protons (billiard balls) gave far more large-angle electron scatters than expected. The results can be explained only if there are heavy, compact objects (the quarks, equivalent to the buried ball-bearings) inside the protons.

This raises an obvious question: if an electron has a quasi-elastic scatter on a quark, should we not see the quark recoiling at a large angle too, leaving the shards of the proton behind? The answer is "Yes, but it is more complicated than that". What obscures the simple picture is a property of quarks that makes them harder to study than most other elementary particles, the fact that a single free quark can never be seen on its own. They are like the poles of a bar magnet — cut it in two and you don't get two free poles, you get two smaller magnets each with two poles. According to the successful theory of quantum chromodynamics (QCD), quarks are always surrounded by a local cloud of force-carrying particles called gluons, which act like the magnetic field around a magnetic pole. If we scatter an electron violently from a quark in a proton, then the quark begins to recoil like a free particle. But when it gets more than about 1 femtometre (10^{-15} m) from the remains of the proton, it stretches the gluon field sufficiently to allow a new quark–antiquark pair to be produced, and the antiquark sticks to the original quark and makes a meson.

In highly energetic collisions, many quark–antiquark pairs are created, producing a jet (Fig. 1) of mesons (see ref. 4 for a qualitative explanation, or ref. 5 for the full treatment). But QCD theory says that the meson with the largest momentum will contain the original recoiling quark. A π^+ meson contains an up-quark and a down-antiquark; a π^- contains a down-quark and an up-antiquark, so by looking at the fastest meson we can infer something about which kind of quark (or antiquark) in the target proton was hit. Experiments that pick out this leading meson for special attention are making use of semi-inclusive deep-inelastic scattering.

That is what the HERMES collaboration[1] has done: they scattered 27.5 GeV positrons (the same as electrons for these purposes) from both protons and neutrons contained

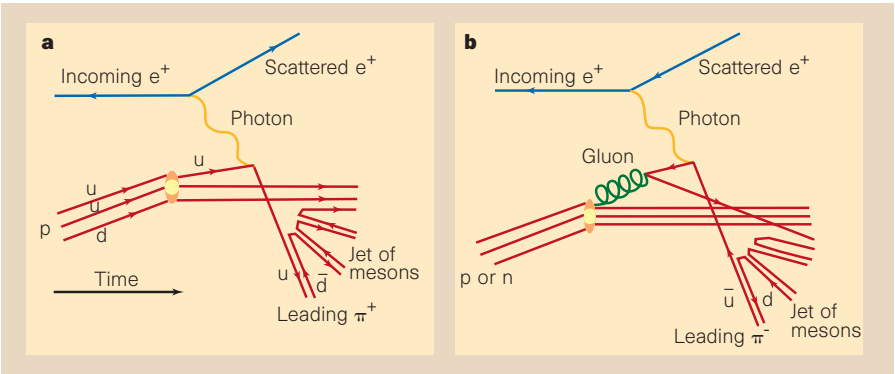

Figure 1 **Principle of the HERMES experiment[1]. a, Feynman graph for semi-inclusive deep-inelastic positron scattering from a valence up-quark in a proton. The exchanged photon is the carrier of the electromagnetic force. If the leading meson is charged, it has to be a π^+ because the recoiling quark is an up-quark. Arrows pointing backwards in time represent antiparticles. b, Semi-inclusive deep-inelastic scattering from a virtual up-antiquark ($\bar{u}$) in the sea, formed by pair production from a virtual gluon. The leading charged meson in this case has to be a π^-, whereas if the scattering were from a down-antiquark it would be a π^+.**