The Discovery and Development of Cyclosporin

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Professor J. F. Borel gave his lecture entitled *'The cyclosporin story – helping to suppress the immune system'* at Fungus 100. This article has been prepared, at Prof. Borel's suggestion, principally from his paper with Z. L. Kis (1991) which appeared in *Transplantation Proceedings*. He has kindly approved it for publication in the *Mycologist*.

Cyclosporin was the first metabolite from any microorganism to be used clinically to regulate the growth and function of a normal mammalian cell. It was discovered and developed by researchers at Sandoz Ltd of Basel, Switzerland. Its current availability to medicine, under the trademark SANDIMMUN, resulted from a combination of chance, systematic teamwork and individual persistence in the face of official financial policy. By 1996, some 200,000 transplant patients relied on its use.

From the beginning in 1958 of the Sandoz screening programme for antifungal antibiotics, it has been usual for Sandoz employees on business trips and on vacation to take with them plastic bags for collection of soil samples to serve as sources of microorganisms.

In 1970, a sample from Wisconsin yielded *Cylindrocarpon lucidum* Booth and one from Hardanger Vidda in Norway yielded *Tolypocladium inflatum* Gams. Both these fungi synthesized neutral, lipophilic metabolites believed to be novel cyclopeptides. Since only *T. inflatum* was capable of growth in submerged culture, this species (Fig 1) was used for the development and large-scale production of these metabolites. The metabolites were not released into the culture media, but required extraction from the mycelium.

Initially, 80 mg of a two-component mixture of metabolites was prepared. This possessed a narrow spectrum of antifungal activity in vitro against, for example, *Aspergillus niger* and *Neurospora crassa*. A larger quantity of some 100 g was prepared for further experimentation, mainly because of its chemical novelty. A subsample was sent to Sandoz in Vienna for testing fungistatic properties in animals. Antifungal activity proved both modest and restricted, but the metabolites, known as 24-556, showed an unusually low toxicity in the animal body and 24-556 was further tested for cytostatic, antiviral and immunosuppressive activity.

Assays for immunosuppressive activity were at that time being investigated by J. F. Borel, because they had been shown on occasion to give falsely negative results with some reference compounds known to be immunosuppressive. This was most fortunate, because although 24-566 was found immunosuppressive by the improved in vivo assay, later evidence showed this activity would not have been found using the earlier assay procedure. No effect was found on tumour cells of mice in vitro, nor on survival of leukaemic mice, these facts indicating that immunosuppression was not linked with general cytostatic activity. This indicated that a really unique metabolite had been found, able to inhibit very selectively the proliferation of lymphocytes but not inhibit proliferation of other somatic cells. Both antibody- and cell-mediated immunity were suppressed and this justified separation and investigation of the two components of 24-566, which were termed Cyclosporin A and Cyclosporin B. These were homologous compounds and Cyclosporin A was much the more active.

By this time in 1973 the stock of 24-566 was much depleted and larger amounts were required for further work. However, the Sandoz management were then re-evaluating research goals. Very large sums of money, of the order of $250 million, would be needed to pursue development of cyclosporin for approval by the US Federal Drugs Administration, yet the potential market, in organ transplantation, was then small. Moreover, there was the possibility that cyclosporin might fail, as ovalicin had recently done. Management proposed abandonment of the project. Those who championed cyclosporin then knew that their only hope was in finding an application for its use in an approved area of research.

This proved to be inflammation. The earlier tests had included administration to rats with

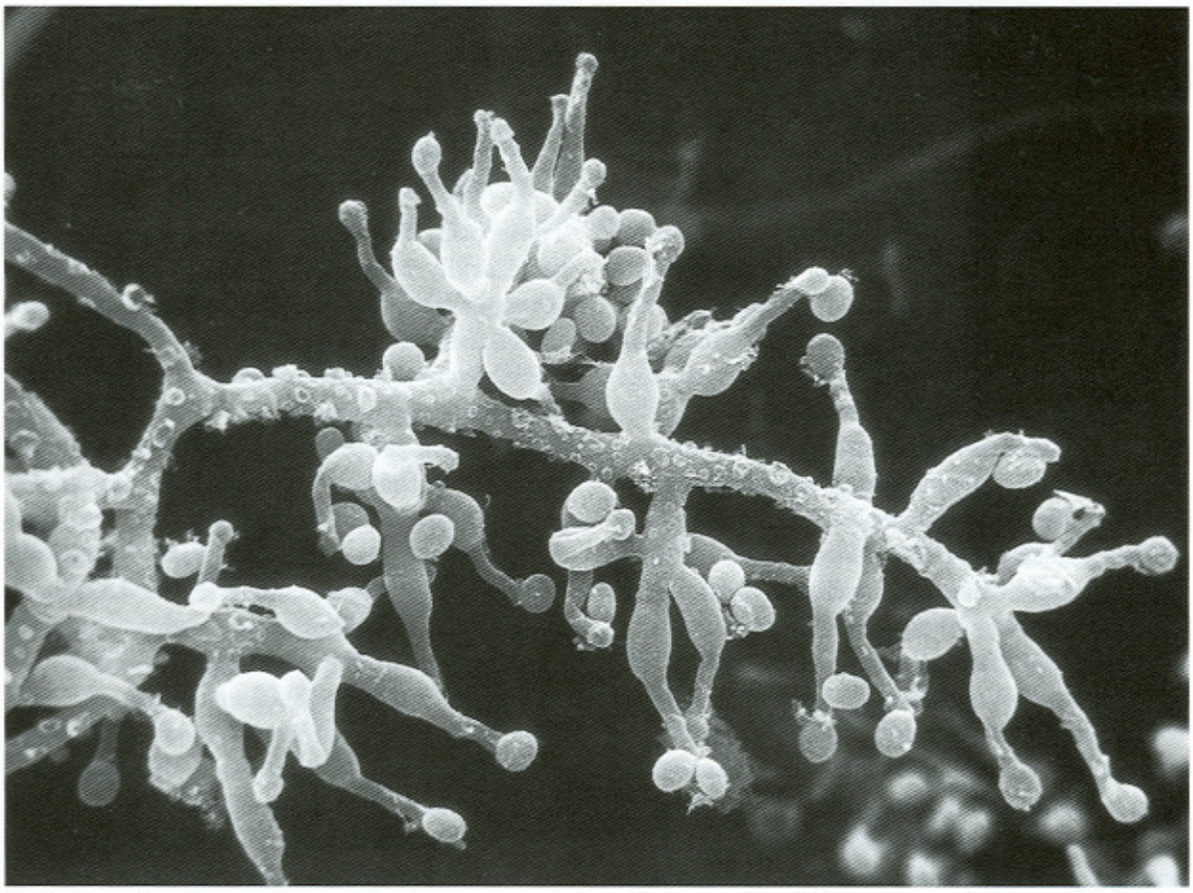

Fig 1 Scanning electron micrograph of the producer strain of *Tolypocladium inflatum* Gams showing conidiophores, phialides and conidia. x 5000 Photograph by courtesy of M. Dreyfuss and U. Strahm, Sandoz Ltd and R. Guggenheim and G. Lüönd, University of Basel (from Borel, J. F., 1983).

experimental allergic encephalomyelitis (in humans, multiple sclerosis), an autoimmune disease model, and the condition of these rats had been markedly improved. This had indicated that 24-556 might improve their condition of chronic inflammation resulting from adjuvant arthritis (in humans, rheumatoid arthritis), chronic inflammation being immune-mediated. Samples of 24-566 were therefore sent to the Sandoz Institute in Bern for H. U. Gubler to test (as a favour!) in the adjuvant arthritis model in the rat. The tests proved strongly positive: there was strong inhibition of symptoms in the immune-mediated inflammatory reaction, either when administered preventively or therapeutically. Moreover, by contrast with other antiphlogistic drugs, ulcers were not induced. Because inflammation was among the Sandoz research priorities, Gubler's crucial report enabled declaration of the project as an official goal. Consequently, cyclosporin was initially promoted to the first formal development in the indication of rheumatoid arthritis – even though its remarkable immunosuppressive properties in the transplantation models were unquestionable. This enabled determination of the chemical structures of the cyclosporins to go ahead.

The structures were determined by Petscher, Kuhn, Lichti, Rüegger, Weber and others and published in 1976, showing that the cyclosporins were indeed cyclopeptides, each made up of 11 amino acids, one of which was new. The second amino acid in Cyclosporin A is alpha amino

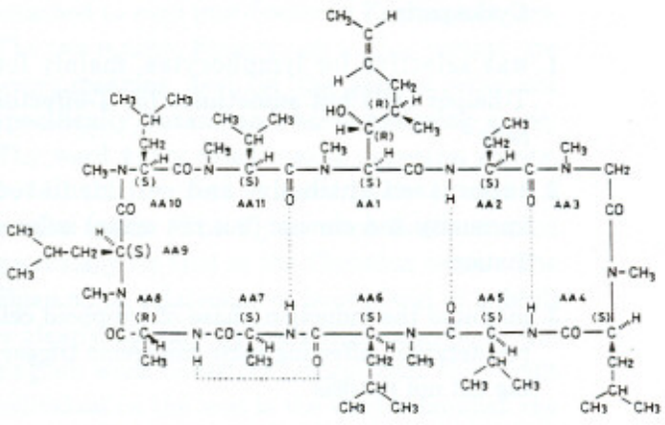

Fig 2 Formula of cyclosporin (from Wenger, 1983).

butyric acid (Fig 2), whereas that in Cyclosporin B is alanine. *Tolypocladium inflatum* produces numerous other cyclosporins, all being composed of 11 amino acids and seldom differing from one another by more than one amino acid. However, of 25 natural cyclosporins and some 750 analogues prepared in the laboratory by 1989 none have shown, in vitro, indications of greater pharmacological activity than that of Cyclosporin A , now known as cyclosporine (in Britain as cyclosporin). The cyclosporin molecule was later totally synthesized by Wenger.

Most of the early immunosuppressive drugs, such as azathioprine, act by blocking all cells in mitosis. This prevents rejection of the incompatible transplant, but at the expense of those body systems with a rapid cell turnover, such as the bone marrow and the cells lining the gut, and leads to severe anaemias and diarrhoea. When tested on spleen and mastocytoma cells in comparison with several of the early drugs, cyclosporin demonstrated a very selective activity on the lymphoid spleen cells, being three hundred times more potent on these than on the non-lymphoid mastocytoma cells. The other drugs (excepting hydrocortisone and anti-lymphoid serum, which have their own disadvantages) were equally toxic to both cell types. Cyclosporin was further almost inactive on bone marrow cell counts and myeloid stem cell proliferation in living mice, whereas azathioprine and methotrexate were causing serious myelotoxicity in patients at the time.

Prior to 1976, knowledge of cyclosporin was confined to Sandoz. In that year a classical paper was published (Borel, Feurer, Gubler & Stähelin, *Agents Actions* **6**, 468) in which the properties of cyclosporin were summarised.

Cyclosporin

1. was selective for lymphocytes, mainly for T-helper cells but sometimes for T-effector cells
2. suppressed antibody- and cell-mediated immunity and chronic (but not acute) inflammation
3. inhibited the induction phase of lymphoid cell proliferation, affecting early mitogenic triggering but not mitosis
4. was not toxic to lymphocytes (its effect being reversible)
5. was effective on all mammals tested (mouse, rat, guinea pig, rabbit, monkey, dog)
6. had no cardiovascular, psychotropic or other pharmacological effect which would seriously limit its effectiveness in man.

These main properties of cyclosporin were outlined orally to a meeting in which Dr David White, of Sir Roy Calne's Cambridge transplantation group, was present. Immediate interest was shown. Cyclosporin was supplied to the Cambridge group and the first animal tests outside Sandoz proved very satisfactory. Calne commented that such clear cut results needed no statistics to make them significant.

Clinical trials were begun in Cambridge and were initially most favourably reported, although by the time of publication some disadvantages were apparent. Initial dosage calculations of 25 mg/kg/d, based on the dog, had been too high and caused moderate to severe, though reversible, kidney dysfunction. The American group led by Starzl preserved renal function by reducing cyclosporin dosage by combination with the steroid prednisone, and this combination has become the baseline therapy in transplantation. Kidney dysfunction remains the principal difficulty in cyclosporin therapy, though is increasingly manageable by carefully titrating the dose for each individual so as to avoid rejection but not to risk renal damage, and by regular monitoring of the drug level in the blood.

Notwithstanding this and some other, lesser, side effects, cyclosporin is accepted today as the first-line treatment in transplantation. Moreover, its potential in autoimmune disorders such as uveitis, rheumatoid arthritis, nephrotic syndrome, psoriasis and others is being continuously developed.

References

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