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History of the discovery of cyclosporin and of its early pharmacological development*

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A major commitment of the pharmaceutical industry is the development of new and efficacious drugs, especially drugs that are effective in indications for which, so far, no therapy exists or which require drugs that have novel pharmacodynamic properties.

The research necessary to achieve such new, designed developments is complex, since the absence of the drugs is itself a reflection of a lack of knowledge in the medical field concerned. The intellectually rewarding approach of deliberate drug design is most difficult due to the default of even adequate hypotheses. The alternative approach is the random screening of innumerable compounds, using the most advanced and sensitive test systems available. However, the chance of discovering a new active metabolite is minute.

The early history of medical immunology is dominated by attempts to induce resistance to infectious diseases by means of eliciting immune responses. Most successful was the development of vaccines. In contrast, several pathological conditions are caused by undesirable reactions of the immune system, be they of „natural“ origin, such as autoimmune diseases and allergies, or the consequences of medical interventions, such as transplantation. The increasing impact of immunology on medical research and practice was becoming more and more obvious. It was in this setting and shortly after the first report on the successful treatment of a human kidney allograft recipient with azathioprine that a laboratory for immunology was established in 1966 by S. Lazáry under the supervision of H. Stähelin, Chief of the Molecular Pharmacology Division at Sandoz Ltd. in Basel [1].

The early development of pharmacologic immunosuppressive agents began worldwide around 1960 and was aimed at the destruction of all rapidly dividing cells with cytostatic drugs such as azathioprine. Later, more selective drugs or procedures were tested and were mostly restricted to the elimination of the immunocompetent cells, namely the lymphocytes. Thus, the lymphocytotoxic effects of steroids, antilymphocyte serum and total lymphoid irradiation were used [2].

H. Stähelin, Head of the former Tumor Chemotherapy Group, had already been testing some antitumor preparations for immunosuppressive activity. Screening fungal products for effects other than antimicrobial activity had been a “program” in his group since 1958 and had yielded a number of interesting compounds, which inhibited proliferation of animal cells, whilst they exerted no or only marginal effects against bacteria and fungi. However, when the efforts in the area of immunology were methodically enlarged, it turned out that a fungal metabolite, ovalicin, was able to considerably suppress the immune response of animals. Proliferation of cells other than lymphocytes did not seem to be affected and, in particular, there was no leukopenia, not even at very high doses. Ovalicin was the first chemically well-defined nonsteroidal product with immunosuppressive activity and no leukopenic potential and may thus be considered a kind of forerunner of cyclosporin (CS). Unforeseen side effects in clinical trials, however, necessitated abandonment of any further use in man [1]. Nevertheless, the experience gained with ovalicin certainly “prepared the mind” (according to Pasteur) for discovering CS and it clearly shows that its discovery was not a case of serendipity.

When I joined the Medical and Biology Research Division at Sandoz Ltd. in Basel in spring 1970, I had the good fortune to take over from S. Lazáry a well-equipped laboratory with good experience in assessing immunosuppressive agents. Together with several technicians I began to investigate in depth the methodology of the two selected immunologic assays used in our screening program for assessing the immunosuppressive potential of natural as well as synthetic compounds. The reason for doing this was the initial remark by S. Lazáry that he felt the in vitro incubation period and also the in vivo treatment schedule used were not optimal, since on several occasions these test models indicated false negative results with some standard immunosuppressive compounds.

Cell-mediated cytolysis was assayed in an in vitro system using spleen cells from mice sensitized with allogeneic tumor cells. The compounds were incubated for a

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prolonged period with the sensitized lymphocytes and their inhibitory effect on the cytotoxic action of these sensitized cells on Cr^{51}-labelled allogeneic target cells was measured. Some important modifications were eventually made which markedly enhanced the reliability of this test model [3, 4].

The other screening assay was an in vivo haemagglutination test in which mice were immunized with a mixture of sheep erythrocytes and L1210 leukemia cells on day O. The compounds to be tested were initially administered intraperitoneally on days 1, 4 and 7 and blood was drawn from the orbital plexus at day 7. An haemagglutination assay was performed with the serum. If the compound prevented haemagglutination, i.e. antibody formation, it possessed immunosuppressive activity. The same mice were further observed in Stähelin's laboratory for the ability of the test compounds to prolong survival, since these inoculated mice would normally die of leukemia around day 16. If a compound prolonged the life-span of such mice, it possessed cytostatic or anti-cancer activity. We made a modification in the treatment schedule – already in late 1970 – that was later to be crucial, because the detection of the immunosuppressive property of CS (formerly agent 24-556) would not have been possible with the old version [5]. In the modified version the mice were treated on 4 consecutive days, i.e. daily starting on day O up to day 3 inclusive. Blood was collected at day 9 (see also Figs. 2-4 in ref. [2]).

It was exactly 30 years ago, in January 1972, that compound 24-556, which consisted of a metabolite mixture extracted from the fungus Tolypocladium inflatum Gams (Fig. 1) and contained mainly CS A and B, was tested in the above screening models (see Table 1 in ref. 6). It turned out that 24-556 failed to suppress in vitro activated cytotoxic T cells; however, it most efficiently blocked antibody formation in vivo in the haemagglutination model. In the laboratory of Stähelin another remarkable finding emerged: 24-556 had no effect on murine tumor cells (P-815) in vitro or on the survival time of leukemic mice, indicating that immunosuppression was not linked with general cytostatic activity. This was in contrast with most of the immunosuppressive drugs before CS, which acted indiscriminately by blocking all cells in mitosis.

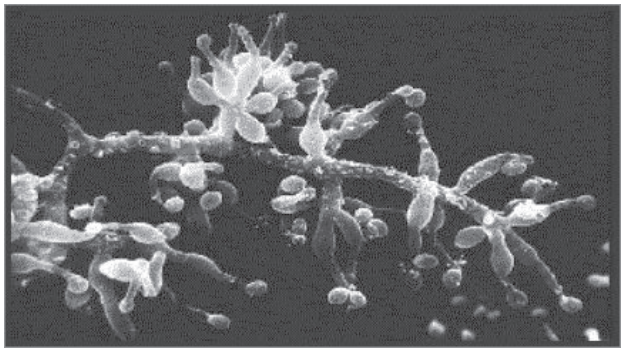

Fig. 1. Tolypocladium inflatum Gams, the fungus that produces cyclosporin A as a metabolite

It is always important to be able to reproduce such key results. Therefore, I instructed one of my technicians involved in the screening to repeat the test and to additionally include a higher dose of 200 mg/ml for oral administration. Unexpectedly, though, the results of the first experiment could not be reproduced (see Table 2-1 in ref. [2]). Although this highly water-repellent compound had been allegedly "dissolved" in a mixture of alcohol-tween 80 and water, as done in the first instance, we discovered only much later, that a dose of over 100 mg/ml can not be dissolved in the above galenic form. Consequently, I persevered and asked for a further repeat test in which the agent was now suspended in tragacanth. In addition, the rather insensitive haemagglutination model was replaced by the much more sensitive plaque-forming cell assay. This third experiment produced a mediocre inhibition of 73%. However, by increasing the dose and the number of treatment, it eventually became possible to reproduce unequivocally the initial potent suppressive activity of 24-556.

A variety of experimental studies followed and we showed that 24-556 suppressed in vivo both antibody formation and cell-mediated immunity, but that it did not induce leukopenia and was not effective in acute inflammation. In view of the numerous failures not only in our laboratory but worldwide, it was almost difficult to realise that with 24-556 we had seemingly stumbled on that rare compound that was able to inhibit very selectively an unknown step unique to the proliferation process of lymphocytes, while apparently sparing the proliferation of other somatic cells. These interesting findings justified the separation of the single components of mixture 24-556.

The next hurdle for CS appeared not in the laboratory but in the executive offices of Sandoz, where it was decided to integrate the company's very limited involvement in immunology into another major field of research. It should be recalled that during the 1960s and early 1970s, immunology had developed rapidly. This increase in basic knowledge had given rise to great hopes, but offered too little in the way of clinical application. The field of clinical organ transplantation was largely restricted to kidney allografts, and most immunosuppressive drugs (azathioprine, methotrexate, cyclophosphamide, steroids) were quite cheap, the exception being antithymocyte globulin. Some of these drugs were also used for treating autoimmune diseases. Finally, there were strong arguments for abandoning immunology because of the failure of ovalicin and because huge sums of money would be needed to pursue the development of 24-556. From the standpoint of research planners, this meant investing in what was then a small, unattractive market – transplantation – with the added risk that the compound might have no clinical value and the company's outlay would never be recouped. Management believed that prospects for a new immunosuppressant were less promising than other avenues of research and proposed abandoning 24-556.

By autumn of 1973, the stock of CS was nearly depleted, and we needed to have larger amounts from the chemistry group in order to pursue our animal experiments. This, however, was impossible without microbiologic fermentation on a larger scale. For the moment, it

seemed we had been brought to a halt. Although discouraged, we few champions of CS knew our only hope was to find an application for 24-556 in an approved area of research. We found it in inflammation. We had earlier shown that 24-556, when given to rats either preventively (developing disease) or therapeutically (established disease), markedly reduced experimental allergic encephalomyelitis, an autoimmune disease model [7]. Because of this important finding, we had suspected that this immunosuppressive compound would also show inhibitory activity in a model of chronic inflammation, such as adjuvant arthritis, since chronicity is immune-mediated. Therefore, Z. L. Kis, the chemist from Microbiology, asked me paradoxically to hand over my very last gram amounts of 24-556 for having it tested as soon as possible in chronic inflammation. He argued correctly: "In your laboratory the compound has no future. Its only chance remains in it showing good activity in inflammation." Somewhat reluctantly, I agreed.

Although the mixture 24-556 had previously shown no effect at all in acute inflammation tests (carrageenan-induced edema), which is not immune-mediated, we suggested to our colleague H. U. Gubler, from the Sandoz Research Institute in Berne, that this compound be exceptionally tested in the more time-consuming, adjuvant arthritis model in the rat. In his 1973 report he described 24-556's strong inhibition of symptoms in this immune-mediated inflammatory reaction, when administered either preventively or therapeutically [8]. It showed a further benefit: in contrast to other antiphlogistic drugs, CS did not induce ulcers. Because inflammation was among Sandoz research priorities, Gubler's crucial report enabled us now to propose the project as an official goal.

Management accepted our proposal. Microbiologists set about producing larger quantities of the mixture, and the chemists were able to supply the two metabolites. Further biologic testing with the single components soon revealed that CS A was the major active metabolite (Figs. 2, 3) [9]. It was named 'cyclosporin A' by the microbiologists, because it is a cyclic peptide and occurs in the spores. Since the fungus produces a whole series of very similar metabolites, these were differentiated by alphabetical letters. Consequently, CS was initially promoted to the first formal development phase in the indication of rheumatoid arthritis, even though its remarkable immunosuppressive properties in the transplantation models were unique [10]. However, it might well have been realized by the experts that the field of autoimmune diseases, including arthritis, was unquestionably a larger indication than that of transplantation.

Early basic work in our immunology laboratory clearly revealed that, in contrast to all previous agents, the purified CS was the very first compound to inhibit the immunocompetent lymphocytes specifically and reversibly, and that it might be considered the prototype of a new generation of immunosuppressive drugs. Indeed, two major studies did exclude possible nonspecific cytostatic effects of CS on cells other than lymphocytes [2].

The effect of CS was also assessed in the tuberculin-type hypersensitivity reaction in guinea pigs. In contrast to the procedure for chemical-induced, delayed skin reactions, the animals were not treated with drugs during the entire sensitization phase, the first drug dose being given only at the time of tuberculin challenge, which followed the sensitizing antigen dose after about six weeks. CS injected just before and just after antigenic challenge considerably impaired the hypersensitivity reaction to tuberculin [7, 11]. This observation made us suspect one possible mechanism of action of CS, namely the suppression of T-helper cell function by inhibition of lymphokine release. It is well known that in this model the presence of specifically sensitized T cells is required, but that the swelling reaction is caused by invading phagocytes which are attracted by lymphokines locally released from these sensitized T lymphocytes.

From 1972 to 1976, only those within Sandoz knew about CS [7]. Now it was time to share this important development. The first publication of our results in 1976 [12] became a citation classic, according to Current Contents 1984 [13]. The other equally basic paper appeared soon after in 1977 [11]. However, it is often forgotten today that the development of CS was at the time a marginal project, even in our laboratory, which was maintained by the special interest of only a few persons.

At the April 1976 meeting of the British Society for Immunology, the main characteristics of CS were presented. In the audience was D.J.G. White from Cambridge, a co-worker of Sir Roy Y. Calne. He immediately expressed

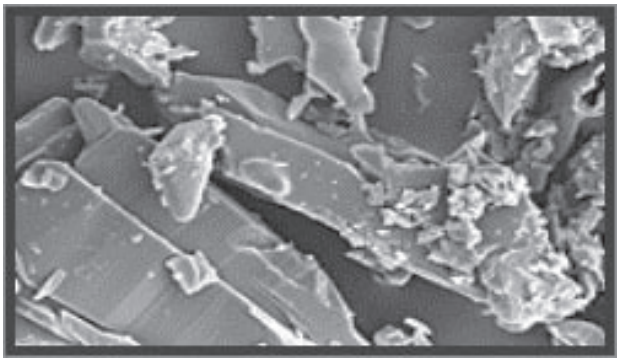

Fig. 2. Electron microscope image of a cyclosporin A crystal

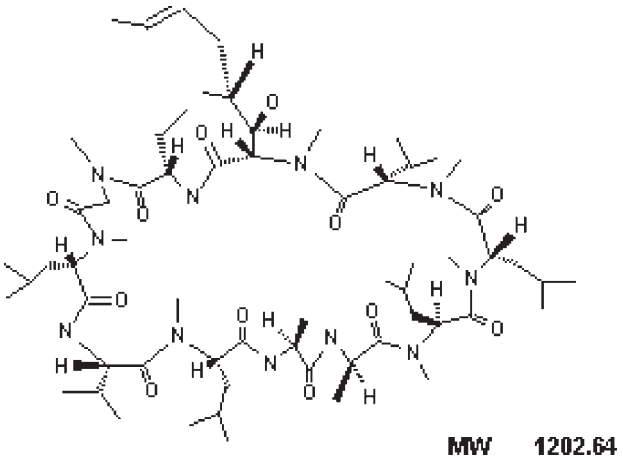

Fig. 3. Chemical structure of cyclosporin A

great interest in the new fungal metabolite, and a supply of CS was shipped to Cambridge where the first animal tests outside Sandoz were performed. Initial results were impressive, especially in orthotopic heart grafts in the pig (see Table 2-2 in ref. [2]). From these experiments, Calne concluded that "Cyclosporin A is sufficiently non-toxic and powerful as an immunosuppressant to make it an attractive candidate for clinical investigation in patients receiving organ grafts" (for references see under ref. [2]). Rapid publication of these findings boosted worldwide interest.

The next step was to study the absorption, distribution, metabolism, and excretion of the drug in normal human volunteers. Generally, studies done in animals must be repeated in humans, in both healthy volunteers and patients. Serious troubles arose at the very beginning of this phase in late 1976. The first time a single oral dose of CS was given to humans (pure, undissolved drug administered in gelatin capsules), it was not absorbed; that is, no pharmacologically active levels of CS were detected in their blood. Yet we remained convinced from our considerable experience with various galenic forms in animal studies that improvement of absorption was only a technical problem. Earlier, our colleague H. Wagner, who had formerly worked on lipid absorption in animals, had suggested dissolving the compound in pure olive oil for two reasons: because of its lipophilicity and because, during absorption in the gut, the resulting emulsion of the olive oil might act as a vehicle to transport the dissolved CS across the intestinal wall. Experimental evidence in animals supported the accuracy of this crucial suggestion [14].

In March 1977, I and two other colleagues (B. von Graffenried and H. Stähelin) volunteered to swallow the drug in three different forms. I myself took the highly hydrophobic compound mixed in a new but efficiently absorbed vehicle, using pure ethanol, water, and some solvent (Tween 80). It was a distasteful concoction that made me feel intoxicated, but two hours later, using two different bioassays, a blood level of 1 μg/ml was measured. Drug dissolved in olive oil produced lower but still significant blood levels, but in gelatin capsules it produced no detectable level [15, 16].

From a marketing perspective, there were still skeptics. Production of the drug proved extremely difficult and required an expensive and elaborate purification process. Because resolution was faltering, someone had to take the lead, create enthusiasm, and encourage further work. Someone also had to correlate our experimental findings with trials outside Sandoz. Consequently, at the end of 1977, I arranged to bring Calne and White's outstanding animal work to the attention of Sandoz management [17]. Our results and theirs at Cambridge were consonant: We had found the prototype of a new generation of immunosuppressive drugs. At the end of that memorable meeting in Basel, management was convinced. Even more vital, it agreed to commit research and development funds and staff to continue testing the pharmacological potential of CS, a decision that opened the way for pilot clinical trials [18, 19].

Finally, I am moving to the very difficult clinical breakthrough. In June 1978, the first patients were being investigated by Sir Roy Calne at Addenbrooke's hospital in Cambridge in mismatched cadaver kidney transplantation and by R. L. Powles at the Royal Marsden Hospital in Sutton in bone marrow transplantation. However, there were some major hurdles to vault before CS's role in medical history would be secured. Calne and coworkers published in The Lancet their clinical work with 34 recipients of cadaveric organs in whom CS had been initially used as the only immunosuppressant [20]. Although this kind of reliance upon a single drug to control rejection had never been feasible before, further clinical trials were questionable, because the incidence of lymphomata was unexpectedly high, none of the kidney recipients had normal graft function, and there was a high patient mortality.

What had gone wrong? Calne had based his first human dosages on those that had been effective and well tolerated in kidney allografted dogs: 25 mg/kg/d. At that time, insufficient immunosuppression was a common problem, so when his patients' kidneys functioned abnormally (or not at all) he interpreted this as an ongoing rejection. Additional immunosuppression was given: prednisone and cytimun, a cyclophosphamide derivative. It was only when he began seeing infections and lymphomata that he realized he had been oversuppressing at 25 mg/kg/d. It was by lowering the dose that he saved CS for clinical use. Retrospectively, we know that high doses may rapidly cause moderate to severe kidney dysfunction.

However, beginning with another key clinical trial in late 1979, Starzl claimed that full exploitation of the drug would be possible only if it was used in combination with other agents, especially prednisone. By using moderate to low doses of prednisone, he achieved a better control of the rejection reaction and, at the same time, preserved renal graft function by reducing the requisite dose of CS [21]. Today, the combination of CS and steroids has become the baseline therapy in transplantation, an approach that has lessened patient morbidity.

After telling the early history of CS, I would like, in conclusion, to quote a sentence from Harry Truman: "It is amazing what you can accomplish if you don't care who gets the credit." Our early, often uncertain research years were marked by nonbureacratic cooperation and tremendous enthusiasm among a handful of such idealistic individuals. The history of CS also illustrates the unpredictable contribution of individual scientists and doctors, preceding events and strokes of luck, logical approach and intuition, and shows the influence of a favorable coincidence of each of these factors on the delicate balance between failure and success [22].

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