0014-4754/96/010005-09$1.50 + 0.20 © Birkhäuser Verlag Basel, 1996

Reviews

The history of cyclosporin A (Sandimmune®) revisited: another point of view

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Abstract. The immunosuppressant cyclosporin A (Sandimmune®) has become the first line treatment for preventing rejection of transplanted organs and for certain autoimmune diseases. The discovery of that drug and its preclinical development are described, and it is shown that most earlier accounts of the history of this compound are, in important respects, incorrect and misleading.
Key words. Cyclosporin A; Sandimmune®; ovalicin; cytochalasin; fungus metabolites; history; discovery.

1. Introduction

The search for metabolites of fungi as possible new drugs was begun at Sandoz Ltd. (Basel) in 1957. This program was initiated and implemented by C. Stoll (cultivation of fungi), C. Tamm (chemistry) and myself (biological testing). A number of new products with remarkable activities emerged from it. One of them, cyclosporin A (CsA), has made its way through pharmacological, toxicological and clinical testing to widespread therapeutic application. It has become a medically and, for Sandoz economically important compound, with sales of more than one billion US$ in 1994, including the new galenical formulation Neoral®. CsA (ciclosporin, cyclosporine, Sandimmune®), is today the first line drug for preventing rejection of transplanted organs. Unfortunately, its history has so far been reported incompletely and, in important respects, incorrectly. It is the purpose of this account to correct this situation and to prevent further copying of mistakes from one author to another.

In section 2, a short outline is given of some highlights of our work with fungal products during the 15 years before CsA was discovered. This constituted a kind of learning period which prepared the way for the discovery and development of CsA. Several tens of thousands of culture broths of soil microorganisms were tested for cytostatic activity in tissue cultures of chick embryo fibroblasts (regarding method, see ref. 68) or in cultures of murine P-815 mastocytoma cells[62]. The active fil-trates of the fungus cultures were then – guided by the evaluations in cell culture – extracted and purified by the chemists. In the early years of the program, only the preparations active in mammalian cell systems were also investigated for antimicrobial activity; later, testing for antimicrobial effects became predominant. Particularly crucial for our later work with CsA were the investigations involving the non-myelotoxic immunosuppressant ovalicin; therefore, the history of this predecessor of CsA is reported more extensively in section 3. Sections 2 and 3 constitute the 'background'. Section 4 presents the amended history of CsA, and in section 5 some of the most serious mistakes and shortcomings of previous reports on this topic are described.

This essay is written from the point of view of a person involved in preclinical pharmacological testing. Aspects of culturing the fungi and of the chemical, clinical and other work involved are therefore only touched upon, although the contributions by microbiologists, chemists and others to the discovery of CsA have, until now, likewise been reported in a biased and inadequate manner.

2. Cytochalasin B, brefeldin A, verrucarin A, anguidine, chlamydocin

Two active metabolites emerging from our program were, due to lack of useful effects in animals, not investigated more extensively by us, but have since become important research tools. Phomin was isolated in 1959 from cultures of a fungus (*Phoma exigua*) and characterized in my report of 1959 as an inhibitor of cell proliferation and of leucocyte motility[60]. Soon after the publication of the structure of phomin[51], Carter's first communication on cytochalasins[20] appeared. It turned out that cytochalasin B[1] was identical to phomin. More recent[45] is the revival of interest in brefeldin A, isolated from *Penicillium brefeldianum*[35,55] at the Sandoz laboratories in 1960 because of its cytostatic effects[61].

Two other fungal products, verrucarin A and anguidine, aroused our interest because of their potent cytostatic activity in cell culture. Since they also inhibited tumor growth in animals, they were tested by Clinical Research at Sandoz in leukemic patients in the 1960s; results were, however, unsatisfactory. Equally disappointing were later clinical trials with anguidine, initiated by the National Cancer Institute of the US[23]. Both metabolites are trichothecenes with an epoxy group. Verrucarin A[32,73],

produced by the fungus *Myrothecium verrucaria*[34], is one of the most potent known cytostatic compounds[53,62] and is also an extremely active antiviral agent[74]. Still stronger is its effect on proliferation of stimulated human lymphocytes[46]. Anguidine (diacetoxyscirpenol) is a metabolite of the fungus *Fusarium anguioides* (later identified as *F. diversisporium*). Its structure was published simultaneously by Flury et al.[29,56] of Sandoz and by Dawkins et al.[22] of Imperial Chemical Industries Ltd. Besides inhibiting cell proliferation, it is also a highly potent antiviral compound[69]. The mechanism of action of anguidine and other trichothecenes, e.g. verrucarins, has been reviewed[24]. On a completely different line, trichothecenes (including diacetoxyscirpenol) have acquired a bad reputation because of findings suggesting their use in chemical warfare ('yellow rain') in southeast Asia[44].

The isolation of the next highly active fungus metabolite, chlamydocin, like CsA a cyclic peptide with a novel amino acid[21], has been briefly described[65]. By making extensive use of a bioassay – a procedure which again became crucial during the developement of CsA – we showed that the cytostatic activity of the peptide is rapidly lost because of inactivaion in the intact animal and in fresh blood or serum[71].

3. Ovalicin, the predecessor of cyclosporin

In 1962, we found that culture fluid of the fungus *Pseudeurotium ovalis* strongly depressed multiplication of P-815 mastocytoma cells. Guided by this cytostatic activity, our chemists isolated the responsible metabolite, ovalicin, in 1965. It was a very potent inhibitor of the mastocytoma system with an IC-50 (50% inhibitory concentration) of 0.3 ng/ml. Though of low general toxicity, the metabolite was unable to increase the survival time of mice inoculated with P-815 mastocytoma cells. Ovalicin is a sesquiterpene (as is anguidine), containing two epoxy groups[5,57].

Since the principal task of our group at that time was the development of anticancer drugs, we had only occasionally tested compounds for their effects on immune responses, e.g. in the hemagglutination test[63]. Mainly due to the initiative of M. Taeschler, then head of our Pharmacology Department, and with the support of A. Cerletti, director of Medical and Biological Research, we decided in 1965 to make immunology a routine part of our pharmacological research. The chemists and microbiologists, foremost among them J. Rutschmann, then head of Chemical and Microbiological Research, were also eager to see their products being tested for as many biological activities as possible.

Thus in January 1966, S. Lazary, a veterinarian who had specialized in microbiology and immunology, joined my Group, and the task of setting up an immunology lab. unit was assigned to him. After he had installed a number of relevant methods and proven their value by testing several drugs with known effects on the immune system[40], I asked him to investigate ovalicin for immunosuppressive activity because it had diminished the spleen weight of treated mice. To our delight, this fungus metabolite turned out to depress the immune response strongly, its activity measured by determining the antibody (hemagglutinin) titer after the mice had been immunized with sheep erythrocytes. The compound was then found active in other tests for immunosuppression as well, such as the Jerne-Nordin assay of antibody-producing cells in the spleen of mice, in experimental allergic encephalomyelitis in rats, rabbits, and dogs, and in prolonging the survival of skin allografts[41–43]. Ovalicin also depressed delayed type hypersensitivity reactions, reduced the immune response in monkeys (S. Lazary, unpublished results) and inhibited swelling of joints in Freund's adjuvant arthritis in rats (D. Wiesinger, unpublished results). The effect of ovalicin on the kinetics of the antibody response was also analyzed[2].

A crucial aspect of the pharmacological properties of ovalicin was its lack of bone marrow toxicity in animals, even in the highest tolerated (non-lethal) doses, and that it did not affect cell division in the intestinal epithelium[41], as do most other cytostatic drugs (see, e.g., ref. 68). Without this specificity for the immune system, ovalicin would not have merited further work since at that time (1966/1967) other drugs with a good immunosuppressive activity – some at lower doses than ovalicin – were known, e.g. amethopterin, cyclophosphamide, azathioprine, but they are all myelotoxic, their dose-limiting effect being primarily leukopenia. Ovalicin can therefore be regarded as the first low molecular weight, nonsteroidal immunosuppressive agent which was non-toxic to the bone marrow; ovalicin was thus the prototype for a novel class of immunosuppressants.

In 1969 studies in humans were initiated. The drug was shown to inhibit antibody formation in humans significantly, but caused a pronounced decrease in blood platelets in several test persons. This side effect, which precluded further trials in humans and may have been due to platelet aggregation, had not been observed in mice, rats, dogs, or monkeys, but was later found to some extent in hamsters.

Another peculiarity of ovalicin is its powerful lethal effect when applied to the skin of guinea pigs[66]. The compound is even more active in T-lymphocytes than in mastocytoma cells. Hartmann and coworkers[36] found an IC-50 of less than 0.3×10^{-11} mol/l (i.e. about 1 pg/ml) for the inhibition of proliferation in mixed mouse lymphocyte cultures, as measured by [^{3}H]-thymidine incorporation. On a weight basis, ovalicin is about $600\times$ and $10\times$ more potent than CsA and FK 506 respectively as an inhibitor of [^{3}H]-thymidine uptake into concanavalin A-stimulated lymphocytes[54,78]. A more recent in-depth study of the mechanism of action

of ovalicin at the biochemical level is unfortunately lacking.

4. Cyclosporin A

At the beginning of May 1970, Jean-François Borel joined my Research Group, which then consisted of about 35 persons and five laboratory units (each of them directed by an academic researcher) and was part of the Pharmacology Department. One of these laboratory units, the one dealing with cancer, was under my direct control. Borel had been doing research mainly in the areas of blood groups and chemotaxis. After having been introduced to his work for two months by his predecessor, S. Lazary, he became head of the Immunology Laboratory in my Group in July 1970. Lazary left the company to return to the university. Borel had, upon his entry to Sandoz, submitted a project in the area of chemotaxis and was permitted to pursue it. He reported to me until the end of December 1978 (incidentally the time of the first publication of the immunosuppressive effect of CsA in transplant patients[19]). We regularly discussed ongoing work either between the two of us or during the usual meetings of the Laboratory Heads in my Research Group. Borel, not having any experience – and, as an agricultural engineer, no formal training – in medicine, pharmacology, chemotherapy or immunosuppression, needed frequent advice particularly during the early phases of work with CsA, and when problems arose.

In order to find new chemical leads for drug development, a so-called 'General Screening Program' was started in January 1970 on the initiative of K. Saameli, then head of Pharmacology at Sandoz. The program was quite comprehensive and consisted of about 50 pharmacological tests, performed in the different Groups of the Pharmacology Department. About 1,000 preparations were fed into this program every year, most of them pure synthetics; a few were, on the other hand, partially purified microbial metabolites. I had decided that my Group should participate in this program, among others, with evaluations related to cancer and particularly immunology. For the General Screening Program I had conceived a novel procedure which permitted the use of the same mice for testing for anticancer activity (leukemia L-1210) and immunosuppression (hemagglutination assay, reflecting effects on antibody formation); this saved on animals, quantity of compounds, and work. Lazary and I showed in November 1969 that the two evaluations did not interfere with each other.

Every week, 20 preparations were fed into the General Screening Program. This was organized by the pharmacologist D. Römer who received the substances from the Chemical Research Department and sent them in batches to the different Groups of the Pharmacology Department. The chemists suggested whether their products should be tested in the General Screening Program or in more specific test systems. One week in December 1971, the batch of 20 preparations sent by Römer included, among a number of synthetic and semisynthetic compounds, preparation no. 24-556 a partially purified fungal product which was later found to consist mainly of CsA. The results obtained in my Group with 24-556 were quite clear-cut: no activity was found except in one test. This assay involved immunizing mice with sheep erythrocytes, preparing an injectable solution of the test compound, injecting the mice daily by the intraperitoneal route with this solution for four days, taking blood 9 days after immunization, obtaining serum from the blood, and titrating it for antibodies (hemagglutinins). The test was performed with preparation 24-556 in my laboratory under the supervision of the experienced technician A. Trippmacher, except for the titration which was done in Borel's laboratory, using a special microtechnique which Lazary had introduced. The titer of the hemagglutinating antibodies had been reduced by treatment with 24-556 by a factor of 1024 in comparison to the controls. I signed the form with these results on January 31, 1972 and sent a copy of it to D. Römer.

24-556 failed to inhibit the proliferation of P-815 mastocytoma cells in vitro and did not prolong the survival time of mice inoculated with leukemia L-1210, thus indicating that immunosuppression was not due to a non-specific antiproliferative activity. In tests of the General Screening Program performed in other Groups of the Pharmacology Department, the only remarkable effects of 24-556 were a weak analgesic activity and a considerable increase in blood urea nitrogen in the serum of rats treated for one week. This latter finding was reported by the Metabolism Group of Pharmacology about two weeks before the immunosuppressive effect of 24-556 became known, and forecast at that early stage the main side effect of CsA: nephrotoxicity.

Preparation 24-556 was derived from a fungus, *Tolypocladium inflatum* (originally classified as *Trichoderma polysporum*), which the Microbiology Group (which was part of the Chemical Department at Sandoz Pharma and not of Pharmacology) had found in March 1970 in a soil sample collected in Norway by the Sandoz researcher H. P. Frey. The microbiologists had cultured this microorganism because of its antifungal effect. (Another producer of CsA had been collected in Wisconsin by E. Härri of Sandoz' Microbiology; the antifungal activity of the Wisconsin fungus strain was detected even earlier than that of the strain from Norway.) The mixture of products of *T. inflatum* was then partially purified by A. Rüegger and colleagues in the Chemical Department – starting from a fermentation culture developed by B. Thiele and E. Härri – and tested in vitro[27] and (at the Sandoz Research Institute in Vienna) in vivo for antifungal effects. However, the activity

against pathogenic fungi was too weak to justify further investigation. In order not to miss any chance, but without any specific expectations, Rüegger and colleagues then forwarded the preparation in November 1971 for the General Screening Program, where it was then given the code 24-556.

After receiving a new batch of 24-556 from D. Römer, I asked Borel to repeat the immunosuppression experiment (production of antibodies against sheep erythrocytes in mice). His results were, however, disappointing: the hemagglutinin titer was reduced about four-fold only (compared to a thousand-fold in the first test, see above) by treatment with 24-556 by the oral or intraperitoneal route, and this despite the fact that higher drug doses had been used in the repeat experiment. This low immunosuppressive activity, had it been found in the initial screening, would not have led to any further testing of 24-556. It turned out later that the poor result of the second experiment was due to the inadequate galenical form used. To obtain a clear aqueous solution, we had made use of dimethylsulfoxide and polysorbate 80 (Tween 80) in the first screening test. The result is given in table 2 of Borel's History[7], experiment No. 1. In the repeat – virtually negative – test (experiment No. 2, ibid.) performed in Borel's laboratory, a drug suspension without organic solvents was used, which was later found to be only very poorly absorbed from the intestinal tract or the peritoneal cavity of the animals. Nevertheless, because of the strong immunosuppressive effect obtained in the first test, we continued the experiments with 24-556. Borel and his technicians then also detected a satisfactory suppression of antibody formation with drug given in suspension by the intraperitoneal or oral route at high doses. Subsequently, activity of 24-556 was demonstrated in a number of other immunological assay[10], assays which had become routine in our Group during the studies with ovalicin.

In the meantime, the culture conditions of the fungus were improved[27]. In 1973, preparation 24-556 was purified and found to contain as the main component a cyclic endecapeptide which was given the code number 27-400 and later baptized cyclosporin A. A minor component of 24–556 was another cyclic endecapeptide of less pharmacologic interest. The structure of CsA was elucidated in a collaborative effort by several Sandoz chemical research laboratories[47,52]. The presence of six N-methylated amino acids and one D-amino acid, and the characterization of the (then new) unsaturated C_9 amino acid, were particularly challenging.

In order to advance further in the hierarchy of compounds, CsA also had to be tested in assays performed in other Groups or Departments and therefore needed official approval by the head of Pharmacology and the Group Leaders. In June 1973, I decided to propose preparation 27-400 for such further development, if it could prevent the symptoms of experimental allergic encephalomyelitis in the rat. I had come to appreciate this experimental model of an autoimmune disease as very useful during work with ovalicin. 27-400 clearly suppressed the symptoms of experimental allergic encephalomyelitis in rats. I also asked Borel to test the compound for leukopenia. As anticipated from the above mentioned screening results, CsA did not significantly reduce the number of leucocytes in the blood of the treated mice. H.U. Gubler of the 'Inflammation Group' of the Pharmacology Department demonstrated in 1973 that 27-400 is effective – i.e. reduced joint swelling – in a model for chronic rheumatoid arthritis, the Freund adjuvant arthritis of the rat. When I presented these and other results at the regular meeting of the Group Leaders in Pharmacology in January 1974, they were received with much interest, and H. Weidmann, then head of Pharmacology, was much in favor of continuing research on this compound.

Further pharmacological studies were then done with CsA; among other things, its activity in bone marrow transplantation was investigated in Borel's laboratory and a beneficial effect on graft versus host disease found in mice and rats. I showed, by measuring [^{3}H]-thymidine incorporation into lectin-stimulated lymphocytes of my own blood, that human lymphocytes are as sensitive to CsA as are murine lymphocytes.

In order to prepare the drugs for testing in humans, the Toxicology Department initiated toxicity studies in 1975. Rats given high doses of 27-400 in the feed for 13 weeks, showed definite hepatic and renal toxicity. In a parallel experiment, dogs were treated with high doses of CsA powder given orally in capsules, but showed no effects. Since the test in dogs was uninformative, another toxicity study with CsA was soon initiated in the fall of 1975, but this time in monkeys. In these animals, the drug exhibited some activity which led to the decision to begin clinical trials.

In the above-mentioned 13-week toxicity study in dogs, the animals were not only tested for adverse effects, but also for immunosuppression; the antibody response of the treated dogs was the same as that of the controls. The apparent absence of effects (including immunosuppression) in dogs made such an impression on Borel that he suggested abandoning further development of CsA. I explained to him that these negative results were most probably due to the galenical form used, and possibly also to some extent to species differences. I then asked Borel to investigate the role of the galencial form for the effectiveness of CsA and recommended to him the use of Tween 80. I had been using this solvent extensively since the 1950's to prepare clear aqueous solutions of hydrophobic test compounds for tissue culture work. I had also noticed that, in some cases, absorption of hydrophobic drugs after oral or parenteral administration in animals is often only satisfac-

tory when a solvent like Tween 80 is used to prevent drug precipitation in aqueous media (see ref. 68). Borel and his coworkers then showed that CsA, dissolved according to my recommendation, exhibited higher immunosuppressive activity as expected, which was apparently due to an enhanced absorption from the gastrointestinal tract or peritoneal cavity.

Physicians in the Clinical Research Department at Sandoz, particularly R. Schmidt, had also become interested in this new immunosuppressive agent and, by late 1974 had begun discussing the possibility of testing it in humans and had contacted Swiss hospitals for that purpose. The first trials in man with CsA were started by B. von Graffenried, an M.D. in the Clinical Research Department, in the spring of 1976. They were complete failures; presumably the compound given as powder in capsules was not absorbed. The clinical studies were then discontinued until absorption from the gastrointestinal tract could be demonstrated. This required the ability to measure blood or serum levels of CsA. Since no sensitive chemical or radioimmuno-assay for CsA was available at that time, I suggested, in 1976, using inhibition of proliferation of mitogen-stimulated mouse lymphocytes in vitro (as measured by [^{3}H]-thymidine incorporation) as a bioassay for CsA in serum. D. Wiesinger in Borel's laboratory subsequently showed that it was possible, using this method, to measure CsA concentrations in the serum of treated animals. Wiesinger had been using the lymphocyte proliferation test for other purposes. In early 1977, B. von Graffenried arranged a comparative absorption study with three different galenical preparations of CsA in humans, in which he, Borel and myself were the volunteers. D. Wiesinger assayed our sera in the lymphocyte test for CsA. She found a strong inhibitory activity in the serum of the volunteer who had swallowed a clear aqueous solution of CsA prepared by the Galenical Department with Tween 80, alcohol and water following my suggestions. A weak, borderline activity was observed in the serum of the volunteer who had drunk CsA suspended in olive oil (a galenical form which H. Wagner, head of another Group in the Pharmacology Department, had proposed), while the serum remained totally inactive after CsA powder was swallowed in capsules. These results paved the way for subsequent clinical studies.

The first report outside Sandoz on the biological activity and the chemical structure of CsA was an oral presentation of a manuscript by Borel, Rüegger and myself, given by Borel at the meeting of the Union of the Swiss Societies for Experimental Biology in April 1976. It was published as an abstract in this journal[13]. This abstract is the very first publication on CsA. The next, a similar report – by the same authors – was an oral presentation given by Borel a few weeks later at a meeting of the British Society for Immunology. A more detailed description of the biological effects of CsA was then published by Borel, Feurer, Gubler and Stähelin in a full paper[10], later complemented by another one (Borel, Feurer, Magnée and Stähelin)[11] in which a selectivity for T (as opposed to B) lymphocytes was suggested.

These results stimulated the interest of some scientists and clinicians in Great Britain, particularly the group of Calne, White and colleagues in Cambridge, and Allison in London. They were given CsA in order to test it for immunosuppression in animals. The results of the Cambridge group with transplanted hearts and kidneys in rats[39], dogs[17] and pigs[18] and the Allison group with rabbits[31], together with our own published findings, convinced them and others that it would be worthwhile testing the compound in humans. In 1978, the British investigators then proceeded to administer CsA to patients with kidney and bone marrow transplants, using parenteral and oral forms of CsA which had been developed in the meantime by the Galenical Department at Sandoz. The first results in humans[19,48] were very encouraging with respect to immunosuppression, although side effects were also noted, and soon aroused an almost worldwide interest in CsA. Further testing of CsA in a large number of laboratories and clinics confirmed the early results and finally led to the introduction of CsA to the market as a drug (Sandimmune®) for the prevention of graft rejection after organ transplantation and of graft-versus-host disease after bone marrow transplantation. For these indications, CsA has become the standard drug. It has also proven its effectiveness in a large number of cases of psoriasis, autoimmune uveitis, idiopathic nephrotic syndrome and severe rheumatoid arthritis, while its use in controlling other autoimmune diseases has still to be explored more thoroughly. The serendipitous discovery of antiparasitic effects of CsA – in malaria[75] and schistosomiasis[15] – has not had any significant medical consequences. The US Food and Drug Administration approved CsA for prevention of transplant rejection in November 1983, about a quarter of a century after our program with soil microorganisms began, and, incidentally, on the same day as the anticancer agent etoposide (Vepesid®), which had been discovered and developed by the same research groups, those of A. von Wartburg and myself[72]. CsA has also contributed substantially to the understanding of immune response. Many steps of the latter are now understood at the molecular level, and the points of attack of CsA have been identified (see, e.g., ref. 80).

5. Shortcomings of previous reports

Most previous reports – particularly the first account[7] – of the history of the discovery and development of CsA are incomplete and, in several aspects, misleading and incorrect. An exception is a more recent communication; after interviewing a number of people involved,

M. Haller presented a well-balanced view concerning the various contributions to the discovery and development of this drug[33]. Earlier publications[65,67] of the present author dealt only with a few aspects of the history of CsA. It is not possible to discuss all the arguments here, but some examples of misleading and incorrect statements in previous reports will be given in the following paragraphs.

In a history of CsA based on an interview with Borel, Bridel[14] says that Sandoz had no experience whatsoever in the area of immunosuppression when Borel began work with CsA. It has been explained above that our Group had discovered and developed an immunosuppressive compound before 1970 (see section on ovalicin) and that all biological tests and the entire biological and technical know-how necessary for the discovery and preclinical development of such a compound had been established in my Group before Borel joined it in 1970, as was the setup which led to the discovery of CsA.

'It so happened that it was I who discovered, in January 1972, the marked immunosuppressive effect of the metabolite 24-556'[7]. Borel repeated this claim in later reports and lectures. As explained in section 4, only the last step of the crucial experiment in January 1972 was carried out in Borel's laboratory, by the technician S. Stutz. She of course recognized the strong immunosuppressive effect, inserted the result, together with the qualification 'interesting', into the screening form, which then came to me for completion and signing. Borel, not being interested in the General Screening Program (his main interest at that time was chemotaxis, for which there was no test in the screening), did not see the result until later[33]. Clearly, to recognize the marked immunosuppression was no scientific feat, once the result was there. It is therefore astonishing that the (supposed) discoverer of this activity has been put on the same level as A. Fleming[83]; the discovery of penicillin by Fleming and that of CsA were two entirely different processes. In addition, as indicated above, preparation 24-556 would have been discarded because of insufficient activity had it been tested first in Borel's laboratory.

'Chance favours the prepared mind'. With this quotation from Pasteur, Borel[7] suggests that it was he who had the mind prepared to realize the importance of the galenical formulation for absorption. This claim has to be judged in the light of the fact that he worked for a long time – until alerted by me – with undissolved and therefore poorly absorbed drug, a fact which almost killed the development of the compound on more than one occasion.

'They [my bosses] ordered me to pour cyclosporin down the drain . . . Several times I was forbidden to work with it. So I worked in secret': Borel in an interview[30]. Neither myself nor any of the 'bosses' on the levels above me ever said anything of the kind. What Borel says here is one of numerous examples (for others see refs. 7, 8, 12, 14, 76) of his unfounded assertion in publications, lectures, and interviews that he had to fight in order to be able to continue work with CsA because his superiors were incapable of grasping the potential of a non-myelotoxic immunosuppressant. The latter contention must be judged by comparing our work described in section 3 above and by considering that, since 1955, I had been extensively confronted with the role of bone marrow toxicity in chemotherapy during my work in the cancer area[64, 68, 72]. In addition, the 'bosses' above me, all M.D.'s with extensive experience in pharmacology, were of course also aware of the importance of the lack of myelotoxicity. That it was Borel himself who explicitly proposed abandoning development of CsA (see section 4) is never mentioned. A few persons involved – those believing the sales estimates from Marketing (see Haller[33]) – were, during some phases of its development, not enthusiastic supporters of CsA. But the steps in developing the compound came in rapid succession, and there are no entries in any protocols from boards enjoying decision competence that mention an intention to drop CsA. This proves that there were no plans or decisions either of my own or at higher levels to delay or stop development of CsA. If progress was interrupted, it was because of unfavorable results, e.g. lack of absorption after oral administration, problems which had to be solved before one could go on. Borel's unsubstantiated claim to have saved CsA from being 'poured down the drain' has earned him a great deal of international recognition, and there is a large body of secondary literature reporting Borel as the rescuer of CsA (see, e.g, Bäumler[3], Bernard[4], Bridel[14], Kolata[38], Re[49], Roberts[50], Woodruff[82]).

'Je fis des essais sur moi-même . . . '[9] (I experimented on myself), 'Borel . . . who himself swallowed one of the first preparations of ciclosporin, before its toxicity was known . . . '[4], 'Borel later was to risk his life . . . By experimenting on himself before cyclosporine's toxicity was known . . . '[30]. These are some examples of grossly misleading reports of the absorption study in humans described in section 4. In reality, this was an experiment arranged by B. von Graffenried with three different galenical formulations of CsA. The trial was planned by the Clinical Research Department, of course after completion of acute and chronic toxicity studies in animals; Borel did not have to beg for it, as he later communicated[14,30]. All three galenical formulations were tested simultaneously, not consecutively, as reported by Borel[7] and Bridel[14]. The composition of the preparation (aqueous solution with ethanol and Tween which I had proposed and not Borel, as he later claimed[30]) which gave the decisive positive result has also been reported incorrectly[7]: the ethanol content was 25%, not 95%. The procedure was, of course, a controlled clinical trial, and not a self-administration ('Selbstversuch') comparable

to the famous test by the Sandoz chemist A. Hofmann with LSD more than 30 years earlier. Self-administration was then (1977) strictly forbidden at Sandoz; regardless of this, even Sandoz officials say that Borel experimented on himself (Wiskott[81]), performed a 'Selbstversuch' (Traxler[77]). All three galenical formulations had been prepared in the Galenics Department, not by Borel, as reported in *Science*[38].

'Borel decided to do a quick test to see if it [preparation 24-556] suppresses the immune system . . .'[38]. This is an example of clearly false statements provoked by remarks of Borel or other Sandoz officials in interviews. Neither Borel nor myself were involved in the selection of preparations for the General Screening Program, and, as also explained in section 4, Borel did not learn of the existence of the fungal product until some time after completion of all screening tests. The Editorial in *Science*[38] is also biased and erroneous in other important respects and has provoked, due to the status of this journal, a number of subsequent incorrect reports. According to this Editorial, 'the hero' Borel not only discovered CsA and 'doggedly insisted that it be tested', but it was also he who had to see if 'compounds isolated from soil samples' had anticancer effects (an area with which he had nothing to do). Similarly one-sided is a later Editorial in *Science*[28]. Other reports are still more misleading, attributing to Borel nearly everything: investigating the soil sample, isolating the fungus, discovering the immunosuppressive effect, conducting tests in humans, realizing the importance of the galenical form, etc.[50].

A further area in which irregular scientific conduct has contributed to the bias in accounts on cyclosporin's history is quotation. In Borel's history[7], not only is the first publication on CsA, an abstract[13] with two other authors besides Borel, not mentioned (Borel[7] describes an unpublished lecture as the first communication on CsA, which is not the case), but *the* original full paper[10] with the biological results regarding immunosuppression and specificity, with three other authors besides Borel, is also left unmentioned. This paper presented the results which were the basis for further interest in CsA both within and outside Sandoz, and it has become a 'Citation Classic' in the Journal *Current Contents* of February 6, 1984. Borel's failure to quote correctly has led to numerous improper citations in subsequent papers by others. Most amazing are Calne's quotations. In his earlier publications (e.g. Calne et al.[19]) he appropriately cites our paper in *Agents and Actions*[10], but later he[16,79] switches to quoting as the relevant publication on early biological studies with CsA a paper[6] in which CsA is touched upon only briefly along with many other drugs, a paper that does not contain the results to which Calne refers, and whose abstract does not mention CsA! One wonders why Calne suddenly decided to suppress the relevant multi-author publication and to quote, instead, an irrelevant one with Borel as its single author.

6. Discussion and conclusions

Much has been published about how inventions or discoveries are made. Drug research is an area particularly abundant in examples both of different kinds of inventions and discoveries, and of various ways in which progress has been achieved. One sequence of intersting findings and developments – including LSD and Hydergin® – which evolved in the course of Sandoz' pharmaceutical research has been reported by Hofmann[37]. In our work described here, we relied essentially on microbial genetic evolution and recombination that has proceeded for billions of years. We made no attempt to use the interesting strategy recommended by De Souza et al.[58], namely that of experimental genetic recombination by fusion of microbial protoplasts in order to produce tailor-made metabolites – despite the fact that I was aware of such possibilities, having been the first to observe fusion of bacterial protoplasts[59].

Many persons have, of course, contributed to CsA, some of them directly, others more indirectly (see also Haller[33]). An example of the latter is S. Lazary. After joining my Group, he established, in a very short time, a laboratory unit capable of performing a number of relevant immunological tests, and he brought with him acquaintance with current immunological research. I myself had had my first exposure to experimental immunology earlier, at a time when the role of lymphocytes in the immune response was not well known, and in a somewhat different area: studies, contrived by E. Suter, on stimulation by tubercle bacilli of a defense reaction in normal and immunized animals[70].

What is the lesson to be learned from all this? Although a single example cannot be the basis for drawing generally applicable conclusions, I nevertheless believe that the course of events which finally led to the discovery and development of CsA is a good illustration of the way pharmaceutical research quite often proceeds. Part of this lesson lies in the following consideration. Cyclosporin-producing soil fungi are by no means rare (they occur in a number of different fungal taxa[25,26]), and many other companies and institutes had been testing large numbers of soil samples for antifungal effects. It is therefore almost certain that other teams running antifungal screening programs with soil microorganisms also had CsA (as an antifungal, and maybe not in pure form) in their hands, probably even earlier than Sandoz, but dropped it because the antifungal activity was inadequate. Thus, apart from the fairly random act of Rüegger and colleagues of submitting the *Tolypocladium* extract for evaluation in the General Screening Program, perhaps the most crucial deliberate step in the discovery and development of CsA was my decision, in 1969, to include a test for immunosuppres-

sion in the screening. Borel[12] also admits that to screen for immunodulators among preparations not selected or synthesized for that activity – where Sandoz was perhaps unique – was a 'basic precondition' for the discovery of CsA. Most biological research steps in the preclinical development of that drug, after the discovery of its specific immunosuppressive effect in the screening, were routine for those with experience in the field.

It is beyond the scope of this account to detail how the one-sidedness in the presentation of the history of CsA came about. But the new facts and points of view given in the present paper on the history of the discovery and preclinical development of CsA may help to determine whether the statement 'The hero of the Cyclosporin story is Jean Borel . . . '[38] is justified.

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