The origins of modern oncology and immunotherapy

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Adoptive immunotherapy

Frequent reports published in the years after the Hiroshima and Nagasaki bombings during the war, of the immediate, secondary and late consequences of the radiation, fuelled the international astonishment and fright already left in their wake. Indeed, aside the mechanical and thermal effects of the explosion, a large number of publications reported the more insidious middle- and long-term effects of the radiation. Among the reported cases were curious states of aplastic anemia, organ failure, debilitating anemia, thrombocytopenia, susceptibility to infectious diseases, and later, a considerable rise in the number of cases of lymphocytic leukemia (until then very rare in Japan). Doctors were above all interested in the aplastic anemia. Reports were being made in the field of irreversible aplasia induced by radiation at doses allowing the destruction of not only certain sensitive tissues, such as intestinal mucosa and lung tissue, but also the complete reserve of hematopoietic stem cells and the fragile network organization of the bone marrow tissue. In other subjects, situations occurred whereby the repair was too long to allow its completion, since the risks of complications were at the time too high. In others still, a steady repair suggested a preservation of medullary zones during the irradiation.

The International Atomic Energy Agency (IAEA) in Vienna, with the help of grants given to research laboratories, proposed to encourage studies on blood restoration following total irradiation; it granted financial support to Georges Mathé for the development of a laboratory dedicated to this subject, which he set up in Paris. Trained in immunology in Bernard Halpern's team, in physiology in Jean Hamburger's team, in hematology in Paul Chevallier's team, in cancerology at the Sloan Kettering Cancer Memorial, Georges Mathé was working on child leukemia in Jean Bernard' s team and was doing research for the Institut National d'Hygiène. His work involved collaborations with notably the English scientist John Loutit and the Dutch scientist Dick van Bekkum, and while exclusively based on laboratory animals, it aimed to achieve both experimental research and clinical goals.

Indeed, far from being exclusively interested in theoretical and abstract subjects, the basis for fundamental research often takes inspiration from real-life issues. While one could reasonably assume on the decreasing likelihood of whole body irradiation of military origin, accidents in laboratories concerned with the exploiting of new energy sources or with the use of irradiation for peaceful and civilian purposes, were on the contrary at risk of increasing.

In 1949, Leon Orris Jacobson and Egon Lorenz showed to the United States that intravenous injection of bone marrow extracts from a donor (D) mouse line, into a recipient (R) mouse line subjected to lethal irradiation, activated in the latter the restoration of bone marrow thus rescuing them from death. They believed they had discovered a humoral factor from within the donor mice, which potentiated the multiplication of hematopoietic precursors in the bone marrow of the recipient mice. Yet, John Loutit and Dirk Van Bekkum demonstrated that this “repairing factor” could be replaced by bone marrow cells. Instead of a hypothetical factor stimulating the proliferation of some stem cells spared by the irradiation, they showed that it was the injected stem cell graft, which multiplied and differentiated to restore the once destroyed bone marrow. Moreover, this graft was obtained with the transfusion of cells that were autologous (from the same animal), isogeneic (from another of the same inbred strain), allogeneic (from another strain), or even xenogeneic (for example cells of rats transfused into mice). All cases led to the rapidly progressive repair of bone marrow function and, simultaneously, its repopulation.

However, the graft of allogeneic cells provoked in the recipient an immune reaction of the donor lymphocytes against the recipient cells and tissues. This would develop into a serious disease of varying intensity but generally fatal, named the graft-versus-host disease (GvHD) characterized by a complex syndrome associating digestive, skin, cardiac, respiratory, hematological and immunological signs. What remained unclear was whether this reaction also concerned the tumor cells, known at the time to be destroyed by non-specific yet strong immunological shock. In other words, it remained unclear as to whether the GvH reaction accompanied reactions of the graft against leukemia (GvL) or tumor (GvT) cells.

In the late 50s, Georges Mathé thus committed himself to researching the potential use of bone marrow trans-

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plants in the treatment of leukemia. In a patient with leukemia already treated by chemotherapy, what remained to be determined were the methods of choosing the best donor, the correct dose of irradiation to precede the transplant, and the intensity of the GvH and GvL reactions.

Mathé started by comparing the conditions of application in humans to the results acquired in animals, in particular the dosimetries in mouse and human and the role of blood transfusions prior to the bone marrow transplantation. The prior introduction of mature allogeneic bone marrow-derived cells into the organism permitted the later transplanted graft to induce reactions conserved in the organism's memory and thus to activate unexpectedly intense and poorly controllable responses.

Before even attempting a graft, Mathé wished to first define the best choice of donor at a time when the complete phenotyping of donor cells was unknown. He discovered that after the injection of a pool of bone marrow cells from non-related donors, the recipient favored the graft of one donor only, the closest matching relative, which induced the most tolerable GvH reaction. This "mixed chimerism", as Mathé referred to it, was until then unheard of and difficult to accept in the scientific community. The controversy was to dampen with the rapidly advancing progresses made in the field, other than simple grafts between twins, towards achieving the goal of characterizing the best donor.

At the time, nobody challenged the dogma of the conditioning necessary by total body irradiation at lethal dose to achieve successful grafting of hematopoietic cells. Yet, this myeloablative conditioning (provoking a total and irreparable destruction of the bone marrow, and thus of all the blood elements present within the organs) induced major immunodeficiency which complicated the rest of the treatment. For this reason, Georges Mathé was considering to transplant bone marrow from "compatible or minimally incompatible" donors after a smaller conditioning (total body irradiation at a dose below 100% lethal), hoping that under these conditions, the GvH reaction would be less intense, that of the GvL preserved, and the post graft immune deficiency reduced. The events that followed made him the first to perform successful bone marrow transplantations on humans that were not identical twins.

At a time when scientists remained largely in the dark in the field of immunology, the bone marrow transplantations they performed were all in animals with nobody daring to attempt one in humans. It was only when confronted with the Yougoslavian radiation victims who had nothing to lose that Georges Mathé dared to be the first to take the plunge. On this occasion, he was contacted in October 1958 by the consultant in radiobiology Doctor Pendic, who worked in the radiocontrol unit of the Vinca Institute of Nuclear Sciences near to Belgrade. On October 15th, the reactor at the centre had suddenly fallen out of control and irradiated six engineers at doses, undoubtedly considering their respective positions and distances from the reactor, that were very high. The dosimetry performed on-site estimated the exposed doses to be between 800 and 1000 rem total body irradiation. Having conducted all the known methods and techniques of microbiological sterilization possible, Pendic asked if Mathé could either come to Belgrade or treat the patients in Paris. He preferred the latter option in view of repeating the dosimetry which Henri Jammet, internationally renowned specialist in radiobiology at the Curie hospital Paris, had committed himself to undertake. Rather than transferring the patients to Professor Jean Bernard's department in Saint Louis Hospital where Mathé worked as assistant, the decision was made to admit them to the Curie Institute where six sterile rooms could be prepared.

The dosimetry performed by Henri Jammet proved to be as grim as that established in Vinca, except for one of the patients who turned out to be notably less affected than the others. American experts reanalyzed these dosimetric data on a phantom. The doses that they telexed corresponded to those that were 100% lethal for one, 75% lethal for four and 30% lethal for the fifth, with the sixth no longer considered at dangerous risk. The correlations of the diverse doses evaluated on the clinical state were closer to those estimated by Jammet than to the other two estimations: they were lower than those evaluated in Vinca and in Paris and higher than the somewhat artificial ones evaluated by the Americans. The doses were in the end established as being from 800 rem for the patient who had manifestly received the most radiation and who died shortly after arrival, to 400 rem for the patient who had received the least; the four others received a dose of 600 rem.

They decided to leave the subject irradiated to 400 rem, to restore his own bone marrow and blood using his own stem cells. The successful restoration of the bone marrow indicated this dose to represent that likely ideal for the conditioning required for organ transplantation. It later served René Kuss to condition the first patient to receive a kidney transplanted from an incompatible donor.

A troubling dilemma surrounded what to do with the four subjects irradiated at 600 rem: either do nothing and await an uncertain medullary restoration, or attempt the first transfusion of bone marrow from an unrelated donor. Georges Mathé decided to play the mixed chimerism card for the incompatible bone marrow transplant. The restoration was of mixed origin, both donor and recipient, in line with the notion of partial chimerism. They confirmed that this induced no notable GvH reaction or hematological effects.

These first tests permitted by fortuitous consequence of an accident that was responsible for a state of medullary aplasia associated with immune insufficiency, had left the patients exposed to major risks of infection. The first risk phase was during the initial period of uncertainty, then during the period of clinical monitoring, and finally after the transfusion of bone marrow during the wait for engraftment and emergence of newly formed blood cells. These patients therefore required protection until complete hematologic recovery. Their fragile state demonstrated that should the bone marrow graft become

a reference treatment, the increased risk from bacterial and viral infection induced by the GvH reaction itself, required consideration.

From here was thus born and developed the concept of a sterile environment for diseased patients, then specific rooms taking all precautions to drastically reduce the diffusion of external germs. In 1964, Georges Mathé set up the first of these rooms in the Fred-Siguier unit of Paul-Brousse hospital. More rooms would follow, at first mostly to isolate patients affected by leukemia in the phase of induction of remission by chemotherapy, then for all phases of intensive chemotherapy for cancer-related diseases. In America, James Holland recognized its huge interest and massively promoted this practice.

Renal transplantations

Georges Mathé worked at one stage with his colleague and friend Jean Dausset in Jean Bernard's department at the Centre Hayem of the hospital Saint-Louis in Paris, on the compatibility between donor and recipient. There, Dausset confided to him that he had found in certain poly-transfused patients, the presence of antibodies agglutinating the white blood cells of other subjects, and had shown him under the microscope the reactions between the white cells of one and the serum of another. Jean Dausset had found in these transfusion antibodies the proof of the existence of groups of white blood cells, just as Karl Lansteiner had revealed groups of red blood cells.

Ruggero Ceppellini to whom Georges Mathé then introduced Jean Dausset, had also observed these post transfusion antibodies and noted their presence not only at the surface of blood elements but also at the surface of all nucleated cells of the organism, underlining the vast field of exploration that was opening up to them. The consequences would indeed revolutionize the notion of self and non-self. Dozens of laboratories seized upon the subject and Mathé was one of the first experimenters to benefit from research made in this field for his own work on bone marrow transplants as well as that embarked upon with René Kuss on kidney transplantation.

During his collaboration with Professor Hamburger, Georges Mathé acquired the certitude that the techniques of extracorporeal blood purification by peritoneal dialysis or artificial kidney, would only ever play a transitory role in the protection of patients against total renal insufficiency and that a better alternative would be kidney transplantation. The dialysis would thus be limited to the waiting period necessary to refine the diagnosis, search for a donor, and prepare the graft. This was in fact also the view of Marcel Legrain and René Küss despite their both being experts on peritoneal dialysis, who therefore asked Mathé to act as consultant in immunology for the renal transplantations that they intended to perform. As it happened, Mathé had experimentally worked somewhat in this field with Jimmy Dempster. Mathé held in high regard René Küss who perfected the intervention technique and practice for the first kidney graft in France, performed in 1952 with Professor Hamburger, pioneer in Nephrology and Intensive Care who designed the first artificial kidney in 1955.

Kuss, Legrain and Mathé asked the radiobiologist and radiotherapist Maurice Tubiana to irradiate the first candidate for transplantation, which he accepted to do at the dose of 400 rem, the effects of which scientists had already been reassured of since the apparent spontaneous recovery of the Vinca patient reported to have received the same dose. Küss communicated the optimal dose of 400 rem to Jean Hamburger and Mathé telegraphed it to John Merrill and Joseph Murray who, in Boston, proposed to graft the kidney of a related donor.

Interestingly for Kuss, Legrain and Mathé, volunteering donors for two of the patients in their care were neither related nor genetically matched. They would therefore be the first to succeed in 1960 and 1961, transplantation of grafts from unrelated donors. Having successfully performed transplantation between false twins in 1959, Jean Hamburger then worked on improving methods of immunosuppression to enable, in 1962, transplantation between cousins.

René Kuss and Marcel Legrain, the inseparable heroes of renal transplantation, would remain friends with Georges Mathé until their death.

Through the increased interest in the press with regards bone marrow and kidney transplantation, Georges Mathé suddenly found himself at the centre of media attention, which he accepted with grace, convinced that it was necessary for France to acknowledge its scientists as well as its does its sportsmen!

Bone marrow transplantation and leukemia

At the beginning of the 60s during a period essentially devoted to addressing questions surrounding organ transplantations, Georges Mathé dedicated a lot of time and effort to the experimental study of the reaction of the graft against leukemia (known as the GvL), which he considered as the next milestone to achieve in humans.

He had shown that recipient mice, injected with a graft comprising a mixture of bone marrow from several donors, biologically chose their donor in terms of relative histocompatibility, ultimately equating to the transplantation from a non-isogeneic but related donor.

Mathé knew that conditioning was necessary to allow engraftment, if only to destroy the bone marrow of the recipient and thus leave the necessary room for graft development. However, it turned out that regardless of intensity, the conditioning would never allow the destruction of the last leukemia cell and thus could never alone increase the rate of success by standard therapeutic means. In other words, after a certain dose of total body irradiation, there would be no therapeutic benefit gained by increasing the dose. Interestingly however,

heterologous bone marrow transplantation in leukemia-grafted animals permitted high success rates due to the development of a reaction of the graft itself against residual malignant cells (GvL). Mathé called this phenomenom « adoptive immunotherapy », in other words how a patient's own immune system could be used to attack the disease. In experimental conditions, the GvL reaction varied in intensity among different mouse lines, donor or recipient, but was particularly efficient when the mouse line K36 received bone marrow cells from mouse line C57Bl/6 as donor.

For a safe and successful translation from laboratory to clinical practice, it would be necessary to exploit the therapeutic effect of the GvL reaction whilst controlling the GvH reaction, the latter of which was responsible for, at the time often unresolvable patient issues. The GvH clinically presents as either acute or, if clinical signs appear after 100 days, as chronic. Signs of acute GvH appear early after injection of the graft and involve the skin, liver, and digestive and respiratory systems. Depending on the intensity of symptoms, the reaction is classed as one of four grades of increasing gravity, each one implicating the implementation of fast and adapted treatment. Chronic GvH essentially involves skin problems (cardboard-like, thickened and squamous appearance) and persistent thrombocytopenia (reduced platelet count).

Georges Mathé discovered in animals that the administration of cytotoxic agents post-transplantation enabled an increase in the frequency of recoveries Indeed, the cytotoxicity displayed by methotrexate and cyclophosphamide against the donor lymphoid cells led to an immunosuppressive effect. An anti-GvH as well as a slight anti-leukemic effect were thus obtained.

Having heard of the theoretical potential of bone marrow transplantation, several parents of children with acute lymphocytic leukemia instantly or secondarily resistant to chemotherapy, for whom there remained no hope for improvement with conventional treatment, turned to Georges Mathé to attempt this as their last treatment option. None of the children had a related donor and an appeal to unrelated donors was required. While the bone marrow transplant initially took in all cases, the succeeding GvH reaction tragically rendered it unsuccessful.

At the time, Georges Mathé learnt from these trials that although immunosuppressive treatment was clearly useful, he remained in the end globally incapable of controlling the GvH induced by the allogeneic graft. To make any progress, he was going to have to reason and discuss each step of the technique: from the dose of conditioning irradiation through to immunosuppressive treatment via cytotoxic agents (optimal moment for administration, combination, dosage).

The first therapeutic failures led Georges Mathé from the early sixties to revisit the multiple donor method, the interest of which he had already demonstrated in animals. It so happened that the recipient the most interested in this method was a young doctor affected by acute lymphocytic leukemia but in remission with strong chemotherapy. In 1963, following total body irradiation at 800 rem, Mathé was able to transplant the bone marrow from six donors genetically related to the recipient. He then witnessed the restoration of bone marrow by cells from the only compatible donor, as revealed by the exclusive tolerance of a skin graft from this donor. What Mathé observed was the phenomenon of acquired tolerance, discovered by Peter Medawar and later confirmed by studies on red cell phenotypes. Mathé had just demonstrated that leukemia was curable.

Among the questions now evoked, Georges Mathé focused on the issues surrounding the conditioning step. What was now established was that the radiation dose delivered had no anti-leukemic effect itself but served to facilitate the tolerance of the graft and the engraftment. Under these conditions, the optimal dose was not necessarily the maximum tolerated dose. Did higher doses in fact themselves introduce a deleterious factor by allowing a state of heightened "cytoadoption" in the recipient and thus an optimized GvH?

With this in mind, Georges Mathé turned his efforts towards performing bone marrow transplantation without recipient cytoablation, by injecting donor lymphocytes to induce tolerance and performing a less-intensive conditioning of the recipient. This enabled a reduction in intensity of the GvH and a reactivation of the GvL. This highly delicate technique, far-reaching for its time, would become more efficient with the increasing diversity of immunosuppressive treatment options.

Destruction of the largest possible number of cancerous cells inevitably coincided with that of blood-forming cells for which preventative and therapeutic solutions were now required. Among the proposed solutions, Georges Mathé developed with Léon Schwarzenberg platelet and white blood cell transfusions and bone marrow transplants. He also had rigorously sterile rooms built, the first of which was in use in 1964 before their adoption in most hospitals worldwide, such as in the department of Professor James Holland at Mount Sinaï Hospital in New York.

American scientists impressed by Georges Mathé's work had already asked that he join them in 1959. Among them, Joseph Ferrebee managed a department in the Mary Imogene Bassett Hospital of Cooperstown endowed with several assistants working on bone marrow transplantation. One of these researchers, a Texan called Donall Thomas, did not believe in the mixed chimerism approach proposed by Mathé. Yet, having performed bone marrow transplantation between identical twins, he joined up with Mathé and Joseph Ferrebee to publish an article on bone marrow transplantation in humans.

In the sixties, Professor Mortimer Bortin approached Georges Mathé with the idea of creating an Advisory Committee for an International Bone Marrow Transplant Registry. Financed by American funds, it aimed to collect the results of transplantations performed by teams worldwide (indications, circumstances in which they were performed, modes of conditioning, nature of the graft, immune-suppressive treatment, observed complications, prognosis). Up to then, doctors remained essen-

tially in the dark with regards the optimum technique. The grouping together of a statistically interpretable number of patients, enabled doctors for the first time definitively validate the indications and the technique. What they were looking for by these means was no longer a failure or a particular success, but survival curves reaching a plateau that reflected a goal rate of cured disease.

A dozen researchers, including Bortin, Mathé, Rim, Santos and Van Bekkum, accepted to share their data with the Registry, thus allowing a profitable cooperation from both scientific and therapeutic points of view with regards to acute lymphocytic and myeloid leukemia. Donall Thomas, however, did not wish to participate towards the Registry; he left Cooperstown in 1963 to work in a department in Seattle, dedicated exclusively to bone marrow transplantation, where he devoted himself to developing the technique through implementation of an important economically and scientifically state-of-the-art platform, which earned him the Nobel Prize in 1990.

The sharing of difficult decisions, risks of failure, and responsibility with the members of the Registry, enabled Georges Mathé to return to his experimental research on conditioning regimens less aggressive to the patients than total irradiation. The results Mathé obtained in trials on conditioning by cytostatic agents were not good. Those obtained in Baltimore by George Santos, who was unable to apply total body irradiation and had thus focused exclusively on such a conditioning approach, were however good. Thus, regardless of method of conditioning, total body irradiation or cytostatic chemotherapy, the desired result was the same: the destruction of the recipient's bone marrow. An alternative to these two so-called "myelo-ablative" approaches would be a non-myelo-ablative approach.

Georges Mathé above all strove to render transplantation possible in humans, notably by maximally reducing the radiation dose in order to reduce the intensity of the graft-versus-host reaction. Thus his European colleagues not only confirmed the type of repair described by Jacobson, but also that this occurred just as well with incompatible as with compatible bone marrow and that in both cases the recovery was due to the grafting of hematopoietic precursors.

Georges Mathé had observed in the radiation-exposed Yougoslavian physicists, that an intermediate dose gave a stable result with no immediately dramatic consequence. He thus envisaged applying a conditioning by irradiation at a lower dose sufficient to allow engraftment and the development of an at least transitory mixed recipient-donor chimerism until establishment of a donor chimerism. In these conditions, it would perhaps be possible to reduce the intensity of the GvH and to preserve and even reinforce the GvL, the latter allowed by transfusion of donor lymphocytes the indication of which would be determined by the evolution of the chimerism. This complicated yet precise strategy would without doubt implicate a more immediate control by immunomodulatory cytotoxic agents.

Among the objections raised by the international community, some researchers argued that only conditioning by total body irradiation allowed the grafting of allogeneic hematopoietic stem cells, and that a dose reduced to 50% lethal allowed the grafting of isogeneic cells only thus reducing the chances of therapeutic success. While Georges Mathé was in agreement with these remarks, he insisted that intermediate doses not only represented a lower risk but also allowed the grafting of both allogeneic cells and isogeneic cells, affording mixed chimerism at the same rate. This mixed chimerism would be confirmed later firstly by Thomas Starzl who, alongside other surgeons, described it in allogeneic liver transplant recipients, and then by other authors including Andréani.

With the arrival of anti-lymphocytic serum (ALS), Georges Mathé thought that he held a specific means of controlling lymphoid cells without altering the proliferation of bone marrow-derived hematopoietic cells. In mice, he showed that this serum eliminated the occurrence of the GvH reaction and preserved the GvL, nevertheless reduced by comparison with that usually observed. While he had thus achieved engraftment with partial chimerism, the concurrent reduction in GvL was in danger of leading them away from their goal of destroying the last cell.

The question was now could the graft itself be improved? In animals as in humans, the donor bone marrow was taken under general anesthesia directly from inside the bone (sternum and iliac). This method thus removed a large number of immunocompetent cells from the donor along with the sought-after hematopoietic stem cells. However, from the mid 60s, scientists knew that such stem cells were present at weak concentrations in the blood and mobilized by certain medicinal agents. Accordingly, they were obtainable by simple centrifugation to serve as grafts the quality of which was better defined knowing the proportion of each cell type. Several European teams confirmed that these grafts allowed hematological repair in comparable timeframes, providing respected donor-recipient compatibility and an existing available donor enabling the decision to transplant.

Active immunotherapy

At the beginning of the 60s, Georges Mathé initiated work to study the concept of active immunotherapy. We all know of the notion of preventing viral and some bacterial infections by vaccination, the aim of which is to inform an individual's immune system of the existence of a specific viral or bacterial antigen so that it reacts promptly if ever it were to meet the pathogen later. At the beginning of the XXth century, Alexis Carrel had shown in the laboratory that a suspension of dead bacterial cells could prevent the later engraftment of tumor cells. At the beginning of the 60s, experiments in mice revealed the efficiency of immune system stimulation prior to attempted transplantation of tumor cells. Freund's adjuvant, known for its strong immunopotentializing properties, increased the quality of the antibody and cell-mediated immune response notably to

weak antigens. However, the secondary effects in animals as in humans disallowed its therapeutic use in its (then) present form (suspension of killed mycobacterium cells emulsified in mineral oil).

Prior to its eventual application in humans, Georges Mathé had tried for different transplanted tumor models and various mouse strains, to determine the optimal approach to stimulating defenses to levels capable, after chemotherapy, of reducing the risk of recurrence. Unable to use Freund's adjuvant to achieve this stimulation, Mathé substituted it with bacille Calmette-Guérin (BCG), a suspension of live-attenuated myco-bacteria. He thus showed that the association of BCG and irradiated heterologous tumoral cells allowed a better prevention of leukemia cell transplantation. Using the particularly aggressive mouse model of leukemia L1210, Skipper showed that the grafting of one single cell was sufficient for complete tumor mass reconstitution, with exponential tumor growth and a doubling time of twelve hours. The preventative effect was assessed using the minimum number of injected cells required to obtain a tumor graft in an animal treated with diverse immunostimulants. This number varied among studies from 10 000 to 1 000 000, thus showing the stimulatory effect of the active preparation against leukemia, one cell of which would have otherwise been sufficient to kill the animal. This minimum number turned out by extrapolation, to be compatible with the number of residual cells in children in complete remission following chemotherapy.

The difficulty consisted in translating these findings into humans, for whom disease treatment imposed several requirements. These included obtaining a state of complete remission by chemotherapy and reducing as much as possible the number of residual leukemia cells without excessively prolonging the duration of chemotherapy. Indeed, too long a duration of chemotherapy increases the risk of selecting a sub-population of mutant and resistant cells, and of weakening the immune cellular defenses.

Georges Mathé proposed to a series of patients with acute lymphocytic leukemia in remission, a specific immunotherapy by allogeneic leukemia cells killed by irradiation, combined with a non-specific immunotherapy by live attenuated BCG. The results of this first study were not statistically conclusive due to the limited number of patients. However, later trials carried out by Robert Oldham at the National Cancer Institute in Bethesda, using the same immuno-stimulatory technique, showed that patients submitted to this form of active immunotherapy presented an amplification of cellular immune responses. Concordantly, Thomas Tursz found a marked increase in the expression of of the human leukocyte antigens HLA-A17 and HLA-B23 in his set of patients submitted to long periods of active immunotherapy, not observed in those submitted to long duration chemotherapy. Finally in 1976, David Machover showed that active immunotherapy secondary to a short cytoreductive chemotherapy gave the same survival rates as a very long and very intensive total body radio-chemotherapy.

The specific stimulation of immune defenses against cancer can theoretically concern targets other than T lymphocytes. Macrophages, granulocytes and dendritic cells play an essential role in the capture and processing of circulating antigens that they then present to the lymphocytes. Depending on the nature of the information on the presented antigen that is transmitted, the lymphocytes then respond by a specific proliferation of specific antibody-producing B lymphocytes or of killer lymphocytes. Various attempts to stimulate in vitro the activity of autologous macrophages followed by reinjection had no obvious therapeutic success.

Other fields of research started to form, concerned with the numerous functions of T lymphocytes (natural killer, regulatory, helper etc.), the mediators (interleukins), and the intercellular cooperation expressed by immune surveillance and tolerance.

Georges Mathé had met Peter Medawar on a number of occasions but remembered above all the one in 1968 in which they had both been invited by Jaroslav Haseck shortly before him becoming one of the first victims of the Prague occupation by Soviet tanks. They had discussed the mechanisms of immunological tolerance. At the time, scientists had distinguished two possible and opposing modes of immune system reaction: rejection, responsible for the elimination of "non-self", and tolerance, responsible for the non-elimination of self. This notion had just been confirmed by Jaroslav Hasek following his observation of skin grafts from one animal species, i.e. mouse, being accepted in another i.e. rats but only if the recipients were newborn; those transplanted into adults were rejected. Peter Medawar confirmed these results in an allogeneic situation in the mouse: he observed a tolerance of skin grafts in newborns in contrast to their rejection in adult mice.

This natural mechanism differs from immunosuppression. The immune surveillance allows individuals to maintain an environment in which their own macrophages capture and analyze enormous quantities of external and self antigens, and, via an adapted response, react specifically against those identified as external and not against the self. The complex mechanism behind tolerance, studied later in the 80s and 90s, involves macrophages and dendritic cells, primary lymphoid organs (thymus and bone marrow), different diffusible mediators and all the effector classes of lymphocytes. Now better understood, this phenomenon offers therapeutic perspectives in the tolerance of allografts and management of autoimmune diseases.

In the 80s, Georges Mathé described in animals a particular form of active immunotherapy known as autoreactive, based on the induction and therapeutic use of auto-antibodies.

With regards passive immunotherapy (use of antibodies aimed against one or more of the antigens presented by the target), he had carried out early work with the Vietnamese chemist Tran Bac Loc. After having immunized rats against mouse leukemia, he fixed onto the antibodies so-produced, a single molecule of methotrexate. The resulting complex had conserved the cytotoxic pro-

perties of the methotrexate, but interestingly now showed superior efficiency as compared to either the antibody or methotrexate administered alone.

Finally, Georges Mathé also established a solid collaboration with Humea Humezawa, a Japanese molecular and organic chemist who had produced several cancerostatic agents. Passionate about the immunotherapy of cancers, he developed an immunostimulant, bestatine, the further development of which Georges Mathé attempted, in vain, to interest the French pharmaceutical industry in.

Today, passive immunotherapy consists of the use of specific monoclonal antibodies and plays a major role alongside chemotherapy in cancer treatment. The modern immunotherapy that Georges Mathé largely contributed to establishing, allows the directing of specific molecules towards specific molecular targets and the in vitro modification of lymphocytic cells destined to fight the disease.

Chemotherapy

As we were reminded in Georges Mathé's opening lecture of January 1967, chemotherapy originated from observations made by the army doctor Alexander that the marines aboard the John Harvey, which was carrying 100 tons of mustard gas and sank during the Bari air raid, all lost their white blood cells. This led to cancerologists embarking upon the study of thousands of cytotoxic substances and their potential anticancerous actions.

Although Georges Mathé is well renowned for his role played in the development of immunotherapy, he also considerably contributed to the development of chemotherapy. He used it to achieve remission before fighting against the remaining cells constituting the residual disease. While his work alongside Professor Jean Bernard was specialized in the treatment of leukemia, he also helped introduce in France the chemotherapy of solid tumors. Indeed, his work was in keeping with screening techniques that he had learnt during his time at Memorial Sloan Kettering Cancer Center. It reflected his overall preoccupation with improving medical treatment of cancer by the development of new medicines, each of which addressed the issues of direct and cross resistance between cytotoxic agents of the same family.

Georges Mathé played an essential role in the development of several important molecules including acriflavine, bestatin, ellipticine, oxaliplatin, triptorelin and vinorelbine. He also determinedly contributed to the development of poly-chemotherapy and of chrono-chemotherapy.

Since the mid 70s, platinum derivatives have occupied a major position in the therapeutic arsenal against cancer. In fact the first available of the platinum family, cisplatin, is responsible for the curing of the large majority of patients with testicular tumors. Many other indications have benefited despite evidence of its toxicity, mainly delayed neurological and renal, which while cumulative is often regressive.

Joseph Burchenal observed that chronic exposure to either of the platinum complexes cisplatin and carboplatin, not only induced resistance of the cancerous cells to the compound used in the treatment, but also a cross-resistance between the two compounds. He also discovered that this resistance did not concern a platinum complex with partially cyclic structure, malonate platinum. Towards the end of the 70s, he asked Georges Mathé to collaborate with him to study a single platinum compound, called second generation, comprising a diamino-cyclo-hexane (DACH) group. They confirmed that when a tumor presented resistance to a first generation platinum compound, such as cisplatin, it did not towards DACH platinum.

Despite its potential, this compound was not a great success. This was perhaps due to a certain hastiness of Georges Mathé who found the compound difficult to solubilize in water or serum, and was unable in his attempts to present sufficient efficacy to warrant its further development for clinical use. Nevertheless, he did come back to this molecule in the 80s while at the Institute of Cancerology and Immunogenetics in Villejuif, with Masazumi Eriguchi, Haïm Tapiero and Huynh Thien Duc, where he assessed its exceptional interest in the treatment of acute myeloid leukemia.

While malonatoplatinum never reached clinical trials, its story does not end there; Georges Mathé often visited Japan where he kept up a number of relations in the chemical synthesis field. He asked the chemist Yoshinori Kidani as well as experts from the company Tanaka Kinkinzoku Kaka specializing in heavy metals, to select a cyclic structured platinum such as that of malonate but which the solubility would be satisfactory. They provided him with a choice of a dozen products of the DACH family and with Yoshinori Kidani and Mazasumi Eriguchi, he studied a compound structurally similar to malonate – oxaliplatin. This proved to be highly soluble and displayed little toxicity yet good anticancer activity against a wide variety of tumors, notably those of the digestive tract. Its action was shown, in both experimental models and upon therapeutic application, to be potentialized upon association with 5-fluoro-uracil and folinic acid, as described by David Machover.

In 1985, Georges Mathé revealed the efficacy of oxaliplatin on most tumors; as with malonate platinum, it presented no cross-resistance with either cis or carboplatin. Its perfect solubility and good tolerance have made this one of the most used chemotherapeutic agents in the digestive tract, notably the colon, a success which Burchenal would have deserved with malonate.

Georges Mathé also worked alongside the two Eriguchi brothers on the optimization of chemotherapy. From the end of the 70s, he focused his attention on finding synergistic effects between different molecules that would increase the antitumor effect. David Machover discovered such an association between the cancerostatic analogue of the RNA base uracil, 5-fluorouracil, and a metabolic potentializing substance belonging to the fo-

late family. Addition of folinic acid to the 5-fluorouracil led to unexpected synergism.

Georges Mathé noted that the folinic acid associated by Machover to the 5-fluorouracil, increased the number of immunologically competent cells. Folinic acid is the active metabolite of folic acid and used as antidote to neutralize the toxic effects of methotrexate without reducing its therapeutic effects. The first clinical trials confirmed the experimental results showing the superiority of this combination over administration of 5-fluorouracil alone.

Georges Mathé then attempted, in the laboratory, to further the anticancerous synergistic effect by adding oxaliplatin to the two products. He obtained a remarkable result, which David Machover successfully translated to humans in the 80s.

From the beginning of the 70s, Georges Mathé also worked alongside Pierre Potier, a highly renowned artist in plant chemistry and director of the CNRS laboratory of natural substances in Gif-sur-Yvette, with whom he devoutly shared the motto "he who dares...". Together they studied extracts of Vinca Rosea, or Madagascar periwinkle. Pierre Potier worked on exotic plants and in particular passionately explored their sap and secretions, extracting alkaloids for later modification by hemisynthesis. In this way, he extracted from the Madagascar periwinkle Vinca Rosea, alkaloids which proved to be particularly harmful to cells in mitosis, representing one of the most important stages of the cell cycle. Mathé initiated clinical trials using vinblastine and vincristine on lymphomas and leukemias. Both these drugs, which made their mark during the pioneering years of cancer chemotherapy, act by inhibiting the polymerization of tubulin that is required for spindle assembly. The development of cancer chemotherapy prevented extending the indications for these drugs. Pierre Potier went on to prepare, by extraction and partial synthesis, vinorelbine which when tested in the 80s by Mathé, proved more efficacious than the two preceding drugs with which it showed no cross resistance.

From remission to cure

Since the beginning of the 50s, Georges Mathé's work was in keeping with an internal logic entirely geared towards cancer therapy. Although the first complete remissions had been achieved against acute lymphocytic leukemia (ALL), all had been followed by a relapse. Mathé understood that curing the disease, whatever its expression (leukemia, lymphoma, solid tumor), would require three steps: firstly, understanding the history of the disease submitted to surgical, physical or medical treatment; then, determining a strategy allowing the greatest possible reduction in the population of residual tumor cells by associating surgery, radiotherapy and chemotherapy; finally, destroying or neutralizing the last cell, the difficulty being to attack the disease when it had ceased to show any clinical or biological expression.

Over many years, Georges Mathé gradually improved Joseph Burchenal's cancerostatic screening by multiplying the number of cellular and tumor models and adding to the original system of tests to recognize cancerostatic effects, tests that could identify virostatic agents and models of immunomodulatory agents. Indeed, cells of cancer patients in remission were less sensitive to the drugs that had previously induced the first remissions, than they were when the drug was first applied. It was deduced from this that these residual cells of the previously treated population, harbored anomalies that determined resistance to the first treatment. Another mechanism of insensitivity of the residual disease resides in their state of latency, in other words, a situation of metabolic quiescence. The chemotherapy, acting only on dividing and thus metabolically active cells, does not affect such cells.

In truth, from the 60s, the new concept of "eradication of the last malignant cell" dominated all others, and it was his determination to reach this goal that drove Georges Mathé towards such diverse research activities. This concept was born from observations made in children in a state of complete remission from leukemia. Whatever the method used to achieve this remission, the suspension of treatment inevitably led to relapse. Two hypotheses proposed to explain this relapse: a recurrence by transformation of normal cells still present or, more likely, a resumed growth of residual leukemia cells. The same problem occurred with solid tumors although the risk for recurrence appeared to be less.

Questions thus posed on the cycling and genetic status of these cells. One hypothesis was that some quiescent cells be present that are resistant to the chemotherapy and susceptible to resume at any moment their proliferative activity. During their proliferative stage, they would in theory become sensitive to the chemotherapy but could also display resistance to treatment through secondary acquired mutations. One could also envisage a mixed status with both quiescent and chemotherapy resistant proliferating cells.

The therapeutic strategy of "maintaining" the state of complete remission, which was hoped would evolve into complete cure, depended on the metabolic status of the residual cell population. Georges Mathé had shown in 1966 on acute lymphocytic leukemia (ALL) in children, that neither the prolongation of induction chemotherapy nor the increase in its intensity, followed by active immunotherapy, was able to cure the disease. A prolonged phase of less aggressive maintenance chemotherapy was necessary to progressively destroy most of the cells, leaving the rest for destruction by the patient's natural defenses.

The analyses made by Georges Mathé concluded upon a mixed status of the residual population. In all cases, the treatment strategy for acute lymphocytic leukemia comprised the successive application of induction, then maintenance, before a potential immunotherapeutic phase. For other tumors, more resistant to the maintenance treatment phase, adoptive immunotherapy by allogeneic bone marrow transplant remained an important strategic element. The adaptation of these strate-

gies to tumors confirmed the validity of the "last cell" concept.

Since the 50s, Georges Mathé had visualized being able to one day destroy or neutralize the last malignant cell. He knew this could only be achieved by rationalizing complex strategies allowing the linking together of different strategies of attack: hormonotherapy for hormone – dependent tumors, chemotherapy for chemosensitive tumors, radiotherapy and active and passive immunotherapy with the use of monoclonal antibodies, all optimally arranged to achieve a cure. This once premonitory vision can be seen in practice today.