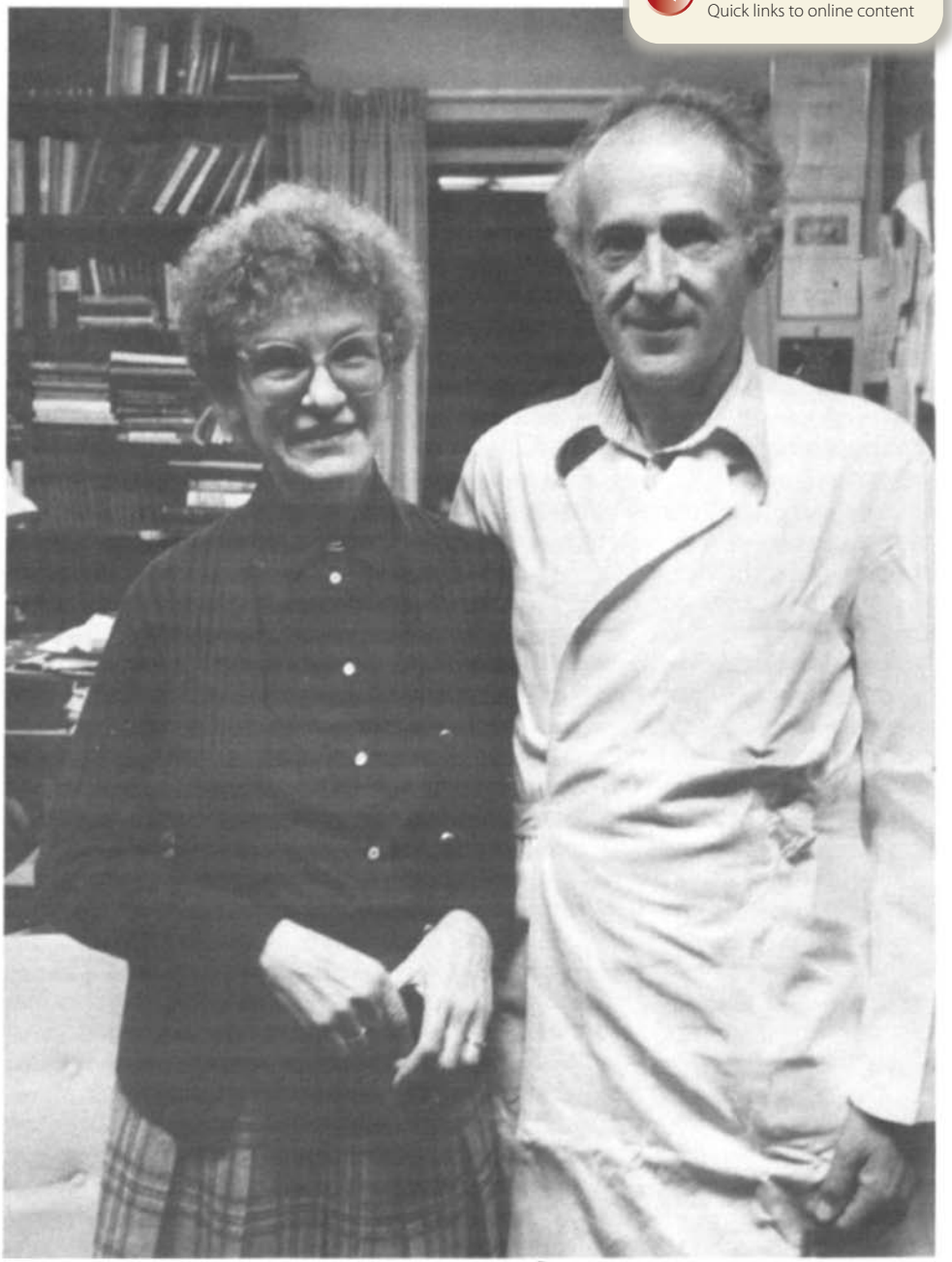

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HOW ONE THING HAS LED TO ANOTHER

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GEORGE KLEIN WRITES:

Dawn

This story starts on the 10th of January, 1945, when I emerged from a cellar on the outskirts of Budapest where I had been hiding, with false papers, during the last weeks of the German occupation. With a totally new feeling about the sunshine that was floating over the snow, the ruined houses, the dead and frozen soldiers, civilians, and horses, I suddenly realized, with a mixture of surprise, guilt, and delight, that I had survived in spite of an 80% chance that I would end my 19 years in the gas chambers or in a military slave labor camp. After a few quick walks in the newly liberated area of the still besieged capital, I decided that it was time to start my medical studies, already delayed by almost two years. During the first year after my graduation from middle school, it was impossible for a Jewish boy to enter medical school. After the German occupation nothing mattered except survival.

We were free at last, but it was a complicated freedom. After a few more days, the Eastern side of the city, Pest, was all in Russian hands. I moved around relatively freely but I was caught twice, like other young men who were automatically regarded as disguised soldiers. In comparison with my earlier escape from a Nazi labor camp, it was an easy matter to run away from the improvised, loosely organized Russian patrols. It was a wise move. Several friends of mine who went out to get a loaf of bread returned years later from Russia.

As soon as the streets were open, I walked to the University to see

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whether it would open its doors for me now. I found deserted buildings, broken windows, and dead soldiers. Together with a friend we therefore decided that we should try to reach Szeged.

The journey of less than 300 km took more than five days. We walked long stretches, hitched on horsedrawn carriages and every other vehicle that we could get on, including a Russian military truck. We arrived in Szeged on February 4. It was a cold and beautiful morning. The city was intact, and we were admitted to the University on the same day. It was a strange place. All the professors had fled to the West. An assistant professor of forensic medicine with a Christlike head and very sad eyes was teaching anatomy, pathology, and forensic medicine all by himself. Students kept arriving from all former theaters of war, labor camps, and illegal hiding. Cadavers were abundant. The large dissection hall of the Anatomy Department was crowded. The smell of formalin, the half dissected or fully prepared body parts, and even the continually tipsy attendant appeared to me as parts of a magic, enchanting landscape, a previously forbidden paradise that was now all mine.

Two years passed as a single wave of febrile activity. I finished three terms during three months in Szeged and returned to Budapest when the university reopened there. I wanted to start research work, but the departments were still paralyzed. They had no resources and the routine work consumed the energy of all staff. Still, I got a first decisive inspiration from the professor of histology, Tivadar Huzella, one of the few internationally known scientists in Hungary and also one of the few true liberals among the medical professors of his generation. In spite of his consistent anti-Fascist stance, and his strong opposition to any form of discrimination during the war, he became a suspected person in the eyes of the new rulers. His uncompromising individualism and his democratic value system invited the enmity of the political opportunists who wanted to see a more compromising person in his position. His arch-enemy, the professor of anatomy, a political opportunist and a scientific nonentity who had resented Huzella's international fame for many years, delivered a list of accusations against him to the "people's court." The sympathies of all the students were on Huzella's side. The crucial trial, where all the absurd accusations—exemplified by the charge that Huzella ate eggs ordered for tissue culture—were readily dismissed, ended in tragedy when the presiding lay judge asked whether Huzella still believed a sentence he wrote during the war. Huzella had stated (an act of great courage at the time) that Hitler, Stalin, and Salazar were equally abominable dictators. If he would have been willing to exempt Stalin and admit his "mistake," he would have been cleared. But he stuck to his words and was summarily dismissed. He died a few years later. Today he has been "rehabilitated."

His home and laboratory are kept as a public memorial. They also house the leading immunological laboratory of Hungary.

Huzella had an exceptional ability to convey his own deep interest in biology to his students. He was convinced that the time had come when biology could be converted from "metaphysical speculation" into a natural science with precision and dignity similar to those of chemistry and physics. He believed that the biology of the interstitial space would turn into detailed biochemistry in a few decades but that the cell interior would remain a black box during the rest of the century. Before blaming him for a lack of foresight, we must realize that most biologists of the time were unwilling to accept his "optimistic" view even about the connective tissue.

I learned some tissue culture, but my practical experience remained rudimentary, and I compensated only slightly by avid reading in the still quite deficient library. After Huzella's removal, I realized that I could not learn more in the now largely nonfunctional department, and so I moved to Pathology. After a few weeks I found myself totally immersed in autopsies. There was a great abundance of cadavers here and very few pathologists. The large postwar classes of medical students had to be taught quickly. I greatly enjoyed the double task of teaching the little I knew and trying to explain to the rushed and often very nervous clinicians what their patients had died of.

In the early spring of 1947, one of "my" students approached me after an autopsy. He said something appreciative about my demonstration and asked whether I would be interested to visit Sweden with a student group. I was amused by his naiveté. Who would not like to visit Sweden? But were we not all aware of the fact that foreign travel was the exclusive privilege of important functionaries and people with much money and many good connections?

He replied that he was currently organizing a trip for students and that he would include me. Hungary still had an elected coalition government at this time. It was possible to get a passport, but this was not sufficient to leave the country. A special exit permit had to be issued by the "Allied" forces, i.e. the Soviet Army. It was very difficult to get this permit, and it was nearly impossible to obtain foreign currency.

I mailed my papers to my student who was interested in Sweden and totally forgot about our conversation.

Decisive Summer

In June 1947 my boss, Professor Baló, told me that I would be responsible for the autopsies during the coming month, virtually alone. I was happy, proud and frightened. I was not yet 22, far from being an MD, but the night's sleep of a professor in surgery could depend on what I was going

to find. The combined feeling of responsibility and awe turned every autopsy into an exciting detective story. During my minimal "spare hours" I also started my first attempts to do some experiments. I was sitting in a corner of the laboratory with a small water bath and a stalagmometer, trying to follow a lead that had been opened up by my chief.

The most important messengers of my future destiny appeared in the shape of two house painters in the middle of July. They had been ordered to repaint the laboratories. I was chased from room to room with my water bath, but I refused to give up. Finally, I was squeezed into a small corner in a tiny windowless alcove that I refused to leave. The painters complained to Professor Baló. With an irritated "you can take two weeks vacation for once" he ordered me to leave my paradise. A senior colleague was to take care of the autopsies. I was angry and disappointed. What was I to do during two whole weeks?

By coincidence I learned that some fellow students, two couples from the Pharmacology Department, were planning to spend the forthcoming week at the Lake Balaton. I was also told that they had invited some other friends and that I was welcome to join them. We were allowed to use the terrace of a bombed summer house and were going to sleep on mattresses, spread out on the terrace. It was quite warm during the first week in August, and we would have a roof over our head. After considerable hesitation, I decided to join them, but I felt ambivalent and uninterested.

The place was unexpectedly pleasant and my fellow students were much nicer in private life than at the University. On the second day, the two other boys went down to the train to meet another student from the Pharmacology Department, who was to join us. I did not know who it was, and since the Hungarian language does not distinguish between *he* and *she*, I did not even known whether we were expecting a boy or a girl. After a while I saw them walking up the hill with the new guest: a dark girl with a strange, breathtaking beauty. I perceived a most unusual combination of hilarity and sorrow, seriousness and play in her eyes. It was Eva, my future wife and colleague until this day.

I had seen her before at the university, but my obsessive preoccupation with work prevented me from giving her or any other girl much attention. Still, I could remember very well how I met her the first time. On the second day of my medical studies in Szeged, I was standing in the Dean's office, to get my papers. She entered, dressed in a skiing outfit, having arrived in the city after a long and adventurous trip from Budapest, like my own. She asked me how to get papers. I saw that she was very beautiful. Her direct way of talking to a strange boy—very unusual for a girl in Hungary at the time—struck me as original and sympathetic. During the forthcoming weeks I saw her at some lectures, but then she disappeared.

Later I saw her name on the posters of the city theater. She was playing small roles in Pirandello and Molière plays. Half a year later I saw her again in Budapest. She had returned to medical studies and came sometimes to my autopsy demonstrations. I knew that she belonged to the same group of students in the Pharmacology Department as my married friends and temporary hosts. Their "gang" treated me with friendly tolerance, and even with a trace of respect for my "knowledge"—in spite of their "objections" to the "dead morphology" that pathology represented in their eyes. I respected their intelligence and their dynamic experimentation and could therefore forgive their blatant ignorance of pathology and clinical medicine.

But this time everything was different. There was one table but only three intact chairs in the ruined villa, and we were six. We had to place a board on each chair to hold two. Eva and I were placed on the same board and had to coordinate our movements to prevent each other from falling down. This trivial problem initiated a contact that metamorphosed after only a few hours into a passion that conquered my entire consciousness with the force of an elementary power. All other interests and problems vanished as if they had never existed. I spent eight days at the lake, intoxicated, overwhelmed, cut-off from all earlier reality.

An unexpected telegram arrived on the seventh day. Everything was settled for the trip to Sweden! My former pathology student or, as we were soon to call him, Our Leader, had succeeded against all odds. He had pursued his plan with obstinate ingenuity and obtained all the exit permits for a group of seventeen students selected by himself with the arbitrariness of a sovereign. We came from different faculties and were to visit Stockholm and Gothenburg as the guests of the Jewish Student Club there, in order to see a country that was saved from the war.

Now I did not have the slightest wish to go. I felt very bitter about having to leave the person who had become more important than anything else in my life so far. The week at the Balaton appeared as an eternity; everything before was unreal. But vague feelings of responsibility and premonition commanded me to go. I left at dawn on a Sunday morning. Eva told me later that she heard the train whistle while half asleep and thought that a beautiful summer episode was now over. She did not believe that I would ever come back from Sweden or that she would see me again.

Cell Biology 1947

The first International Congress of Cell Biology had just terminated when I arrived in Stockholm. I was told that Torbjörn Caspersson was one of the most important figures at the Congress. His recent development of ultraviolet microspectrophotometry on fixed cells created much attention.

The method was based on his doctoral thesis, written in 1936 in German and largely unavailable to English speaking readers during the war years. It was the first major attempt to combine morphology and cytochemistry. Cells were photographed in monochromatic UV light under standardized conditions. A semiquantitative method was developed to map the localization of nucleic acids and proteins in different cell types. Jack Schultz, one of J. H. Morgan's last disciples, was the first American geneticist who saw the potentialities of the new approach. He traveled to Stockholm to work with Caspersson shortly before the outbreak of the war. He brought genetic thinking to the biophysically oriented group. His studies with Caspersson on the banding patterns of polytenic insect salivary gland chromosomes gave the first information about the distribution of nucleic acids and chromosomal proteins and set the conceptual basis for the development of the chromosome banding technique by Caspersson and Zech three decades later.

The chemistry of the genetic material was still unknown at the time of the Cell Biology Congress in Stockholm. Most biologists believed that only proteins could provide the necessary diversity. Nucleic acids were considered as repetitive, boring molecules. Levene and Bass pronounced the death sentence on the coding capacity of the nucleic acids already in the 1930s. The mistaken analogy between the "4-letter alphabet" of the nucleic acids and the phonetic alphabet served as a roadblock: how could one build a language from four letters? Caspersson's semiquantitative measurements of nucleic acids and proteins in different cell organelles led him to conclude that there was a definite relationship between nucleic acid and protein synthesis and that the former might actually govern the latter. This visionary insight was widely disbelieved, however. The idea that nucleic acids might carry genetic information that could be translated into proteins was totally foreign, even to Caspersson. The fundamental discovery of Avery, McLeod, and McCarthy on DNA-mediated transformation in Pneumococcus, published in 1944, was widely ignored or discarded as an artefact.

The Cell Research Department of Karolinska Institute had just moved to the newly built campus on the northern edge of the city; there I was to spend all my scientific years, up to the present day. I visited it first in the middle of August, 1947, the peak of the vacation season and soon after the Congress participants had left town. Members of the Department who happened to be in town were frantically trying to get settled in the new building. As I made my entry, tall, blond, 37-year-old Torbjorn Caspersson was lying under a large instrument in a blue overall, trying to fix the wires. I thought that he was an electrician or a technical assistant. His identity was not revealed to me and I was not introduced to him. After I had

learned the difficult art of protecting him from uninvited visitors a few years later, I could understand the reasons. In 1947, I was desolate when I had learned the next day that he had left for the USA. Only after a long series of complications did I get in touch with him, several weeks later. But my first conversation with him was decisive. Thanks to the rudimentary and largely theoretical knowledge of tissue culture, acquired in the Huzella laboratory two years earlier, I got the best-paid job of my life (if the importance of the salary is considered). I was employed as a junior research assistant, on 500 Sw Crs (about US $100) per month.

I still remember the mixture of ecstatic happiness and enormous anxiety. My situation appeared totally hopeless. I knew virtually nothing. I was halfway through my medical studies, still far removed from an MD. I was desperately in love with a girl whom I had only known during a summer vacation of eight days and who was on the other side of an increasingly forbidding political barrier. I did not know a word of Swedish. Still, I was firmly decided to resist the more comfortable possibility of continuing my studies in Hungary.

My motivation was reinforced by a series of articles that kept appearing in the major Swedish daily, *Dagens Nyheter*, translated for me by my temporary host. The Prime Minister of Hungary, Ferenc Nagy (not to be confused with Imre Nagy) of the Smallholder's Party has just fled to the West, and he gave a series of interviews to the Swedish paper. In contrast to the rosy optimism that prevailed among my friends in Budapest who hoped that Hungary would become a democratic country, Nagy's statements had an ominous ring. He said that the influence of the Communist Party was increasing continuously behind the scenes. The Stalinist party leader, Rákosi, was acting under the protection of the Russian forces. The politicians of the other parties were frightened. Several of their leading representatives were arrested on false charges and deported to unknown destinations. Those who remained were increasingly inclined to give in. The police were infiltrated by party members. Nagy did not have the slightest doubt that a Communist takeover was imminent. Similar signals reached me indirectly from one of my teenage idols, Nobel Prize winning biochemist Albert Szent-Györgyi. He was still holding many high posts in Hungary at the time, but he had told his nephew, who was a friend of mine, that the days of freedom were numbered. If you were young and wanted to have a future in science, you should get your degree as soon as possible and leave the country.

Farewell, My Native Land

In mid-September, I decided to go back to Budapest and try to get out for good. My most important acquisition was safely tucked away in my breast

pocket: a re-entry visa to Sweden and a labor permit for continued work in Caspersson's department. My passport was still valid for a few months.

The reunion with Eva confirmed what we both knew already: we wanted to live and work together. The day after my arrival, some of our friends gathered at my home to hear the latest news from the "great world." I told them about Nagy's report and the iron curtain that was about to descend over Hungary. The reaction was mixed. Those who were already preparing to leave believed me. Others wanted to stay and hoped that my report was exaggerated. One of them—still a good friend today—declared that I was probably right, and for that reason, he was going to break all further contact with me. This was his country, Hungarian was his language, his historical roots were here. I should leave, if I felt so inclined, but he had to stay and do the best he could. Today he is the foremost medical historian of Hungary.

I had none of his historical perspectives. I had only one goal, to get married and leave the country.

But how to get married? It had to be in secret, because nobody would understand why two 22-year-old students who had known each other for only a short time and had no income would want to get married. And how could my future wife join me? She had no passport and the difficulties in getting one were now increasing day by day. We agreed that I would go back to Stockholm before my own passport expired and try to obtain letters of invitation for Eva that could help her to get a passport.

The last weekday before my trip was a Friday. Eva and I met outside the pharmacological institute to go to the day's lecture. I suggested that we should go to the prefecture instead and ask how one gets married. We got a list of the many documents you needed. It looked hopeless. It would take months to get them. I suggested that we ask for the first document, a certificate to show that we had no police records. We went to the police station. "It takes at least three weeks." Suddenly I acted on impulse. I had always heard others tell of such things but I myself had neither seen nor done it. I pulled a fairly modest bill out of my pocket and put it in the policeman's hand. "Pardon me, how much time was it, you said?" "I'll go and get it at once," he answered.

It was now 11 AM. We continued from office to office.

Everywhere the same answer: one week, four weeks, six weeks. A little bill in the hand—the certificate was completed within a few minutes. I was amazed to find that the shyness I usually exhibited before persons of authority vanished completely. I learned a lesson about the importance of motivation and the unsuspected possibilities it may open to surpass one's limitations.

By 3 PM only one document was missing: a medical certificate that neither of us had venereal disease. The tests would take several weeks. What to do now?

We went to a slightly older colleague who had recently finished his medical studies. He had just started his first assignment in the Children's Hospital. We told him, in the strictest confidence, about our situation. He had a good laugh and wrote the certificate on the hospital stationary. By 4 PM we were at the prefecture again. We had all the papers and wanted to get married that second. Two other friends, sworn to the highest secrecy, came along as witnesses to the wedding. The official had just finished the day's work and had taken off the broad Hungarian tricolor from his corpulent chest when we rushed in. We heard him telling his wife on the phone that he was on his way home for dinner. Marry us at this time of day? Not a chance! Come back on Monday!

I started to appeal to his human feelings. I had to leave the country on Sunday. How could I leave my young bride alone if we didn't get married? He was noticeably irritated and doubted that we had all the papers. While leafing through the documents, he caught sight of the doctor's certificate that had been drawn up at the Children's Hospital. He laughed until tears ran down his cheeks. This was the funniest thing he had seen during his whole time in service. Now he was in splendid spirits. The flag resumed its place on the large body. We promised to love one another til death us did part.

Afterwards we ate our wedding dinner on the hall bench together with our witnesses. There was only one dish: my mother's carefully packed goose liver sandwiches. In the evening we went back to our parents' homes where no one suspected anything.

That Sunday I returned alone to Stockholm. Eva joined me, after many complications, in March 1948, after the Iron Curtain had already descended over the country.

GEORGE AND EVA WRITE:

The Genetics Congress

In August 1948, several months after we were happily settled in our rented room and Eva had also started to work in Caspersson's department, the International Congress of Genetics took place in Stockholm. The presidential address of J. H. Muller was a scathing denunciation of the abuse of genetics in the Soviet Union. The scientific world was still largely unaware of the fact that the "theories" of a charlatan, Lysenko, had been declared "official" by the Central Committee of the Communist Party, meaning that it became essentially illegal to do any scientific work in

genetics. Muller himself had been the first to introduce Drosophila genetics into Russia, and he was still a member of the Soviet Academy of Sciences at this time. He called Lysenko "a paranoic and half educated young demagogue who had done some work in raising plants but who was in fact ignorant of scientific principles and incapable of understanding them." He added that many of the outstanding Russian geneticists had disappeared, and some had lost their lives in unexplained ways. His speech ended with his resignation from the Soviet Academy. The reaction from Moscow came the day after. They refused to accept his resignation and expelled him.

On the last day of the congress, the Bulgarian delegate asked to make a statement at the concluding plenary session. Speaking in the name of the delegates from Bulgaria, Roumania, Poland, and Czechoslovakia he delivered a strong protest against Muller's introductory speech that was "ill-suited to favor international understanding." His protest was taken to the protocol.

After the session was closed, the representative of Hungary came to us. He did not understand English well, and we had previously helped him. He wanted to know what the Bulgarian delegate said. When he heard our interpretation he became extremely upset. It was typical for the Slavic delegates to leave out the Hungarians! He had to join the protest, he had to think of his family! How could he return without having signed it! But he was out of luck, the congress was over, nothing could be done for him. His panic showed us how the fear imposed by Stalinism had descended on the country we had left only a few months earlier. It was also a reminder of the eternal strife among the nations that have risen from the ruins of the Hapsburg monarchy.

During the Congress we learned about the startling progress in microbial genetics. Bacteriology had been the last citadel of Lamarckism. At this time, when proteins were regarded as the vehicles of genetic information, when notions about a bacterial nucleus were regarded with great suspicion, induced enzyme adaptations and drug resistance were widely attributed to the inheritance of acquired characteristics. But the rapidly growing evidence of clonal variation and Darwinian selection was now definitely gaining ground, integrating microbiology with the rest of biology. Our knowledge about cancer was rudimentary, but we started nevertheless wondering whether the population dynamics of microorganisms and the phenomenon of cancer might share some common denominator(s). Cancer cells are resistant against growth control of the organism. Can they be compared to drug resistant microorganisms? Could cancers also arise by a series of mutations? These seemingly puerile notions were to play an important role for our work later on.

The Cell Research Department

Back at the laboratory, we found ourselves in an exciting environment but facing another impossible situation. We were still medical students in mid-course. We were struggling hard to get into a Swedish medical school and finish our studies. At first, this looked impossible but eventually we succeeded, one by one, taking turns between the school and the lab work. Worse than that, the project given us turned out to be quite unmanageable. Caspersson's methodology was based on the absorption of monochromatic ultraviolet light in fixed cells. Shortly before our arrival, it was heavily criticized by Barry Commoner and other biophysicists. They suggested that the loss of UV light registered by Caspersson's optical system was not due to absorption but to light scattering from the denatured proteins. Due to this artefact, part of the nonabsorbed light would never reach the objective, leading to false conclusions about the localization of nucleic acids and proteins. Their distribution in living cells could be totally different from the pattern suggested by Caspersson's measurements.

Our task was to measure light absorption in living cells. But this was more easily said than done. Tissue culturing of the times followed the dogmas laid down by Alexis Carrell. The plasma clot and the embryonic extract were regarded as essential substrates. Nobody in his right mind would have thought of culturing cells directly on glass, even less on quartz slides. The plasma clot was not transparent to UV. It turned out that my sudden and unexpected employment after my first conversation with Caspersson was due to the fact that I had some experience of growing cells on collagen, Huzella's favorite method. Collagen is poor in aromatic amino acids, and it was therefore expected to provide less of a problem for UV-microscopy.

We struggled frantically to obtain some results. We had no experience, no assistance, and virtually no apparatus. The large UV-equipment was not suitable. The cells were killed by UV long before we could take a picture. Washing and sterilization of the glassware, preparation of the embryonic extract, and most difficult of all, collecting plasma from the carotid of our single rooster with a primitive paraffin oil canule were a neverending struggle. At last, we managed to take a few pictures before the cells died, but we were still far from the number of monochromatic exposures needed for a spectrum. Our future looked dim. Salvation came in the form of two unexpected events. The first was a lecture given by Hans Lettré of Heidelberg on the Ehrlich ascites tumor, which he used for biochemical studies. Eva immediately pointed out that we might obtain homogenous populations of living cells from the peritoneal cavity of the mouse, without having to do any tissue culture at all! Our first attempt to

propagate the tumor, kindly sent by Lettré in the form of a single mouse, ended in total failure, however. The first of our inoculated mice developed a nice round belly that turned out to carry a lovely litter of eight, instead of the expected tumor cells. But the second mouse developed a tumor, and we were in business. But as we were getting ready for the UV pictures, a paper was published by Brumberg & Larionov in the USSR. They used a new, reflecting optical system that avoided the killing of the cells during UV-exposure. They had done all the experiments that we had planned and showed that Caspersson was right and the critiques were wrong. UV-microscopy did measure nucleic acids, and they were localized exactly in the organelles where Caspersson had found them in fixed cells. Our project had become obsolete overnight. What were we going to do?

We expected the worst, but Caspersson suggested that we continue to work with ascites tumors and try to formulate our own project. The early experience of the Genetics Congress came to our rescue. Why was the Ehrlich ascites carcinoma unique? Why could other tumors not be propagated in this freely dissociated "fluid" form? Did most tumor cells require a solid substrate and/or the microenvironment of a solid tissue? We had some ideas about how to start looking at this, but our mouse and tumor facilities were very limited. Inbred mice were totally unknown in Sweden at this point.

Salvation came again unexpectedly. In the summer of 1950 we participated in the International Cancer Congress in Paris. The week was occupied by frantic and hopeless efforts to get acquainted with the entire cancer field, interspersed with meetings with old friends who had left Hungary after us. At the end of the week, we felt definitely reassured about two earlier, disparate but equally important, conclusions: (i) It was very fortunate that we had left Hungary in time, for the Stalinistic system now had a firm grip, and (ii) tumor cells could be definitely regarded as genetically heterogeneous populations with extensive subclonal variation. We also felt proud as contributors to the Congress. George lectured in broken and very slow English in the 32nd parallel section, the late afternoon of the last day, with six persons in the audience (1). It turned out later, however, that this was an extremely important event. One of Otto Warburg's assistants had been in the audience and brought home the great news that the mouse ascites tumor cell is an equally good tool for large-scale experimentation on such relatively homogeneous cell populations as the famous Chlorella algae of the Great Master. Warburg immediately requested the cells and was later very helpful in supporting us. In a letter written in 1956, he stated that we had made a very important contribution, because we had sent him the cells that made it possible for him to solve the cancer problem. All bureaucrats were deeply impressed!

During the Congress week, we also enjoyed frantic, colorful, decadent, exhilarating, and slightly putrescent Paris. Sitting at a cafe on Boulevard St. Michel the evening of Bastille Day we exclaimed: "How wonderful! how crazy! how can one possibly live in a sterile country like Sweden?" Rattling home a week later in a third-class wagon across strike-torn Belgium we said: "How marvelous that we can return to quiet, boring, aseptic, polite Sweden with its thousands of lakes, endless forests, and luminescent nights!"

We had hardly opened the door to our rented room in Stockholm when I saw the new miracle: an express letter in Caspersson's own handwriting. "Get in touch with me immediately on arrival."

One of the main private research foundations in Sweden, established in the memory of Knut and Alice Wallenberg, had asked Caspersson to choose two young men for an urgent mission. They were to go to the United States for several months and report about recent advances in cancer research. Caspersson chose one of my older colleagues and myself. But Eva had to stay home—there was not enough money. My colleague was to travel around from center to center. My task was to work with Jack Schultz at the Institute for Cancer Research in Fox Chase, Philadelphia, on my own project, and to make short visits to some of the major centers in the neighborhood.

We received the news with a mixture of joy and sorrow. It was a fantastic opportunity. But the sorrow and anxiety of being separated again from my young wife, and for quite some time, were further aggravated by the sudden outbreak of the Korean war with its forebodings of a possible world war. We shared our vision of an approaching Apocalypse with most other survivors of the Second World War and the Holocaust. Our officially stateless status added fuel to the nightmares. Still, I knew I had to go.

The Statue of Liberty

The Institute for Cancer Research has developed from a small private research group at Lankenau Hospital, due to the great foresight of Stanley Reimann. When I got there, they had just finished a major expansion from a small group of scientists to a large research center in a magnificent new building. Several prominent biologists had joined the laboratory. The leitmotiv was to look at the cancer problem from the biological point of view. My own boss was Jack Schultz, a lively little man in his sixties. Jack exuded boundless curiosity, joy of life, and great human warmth. He received me as if I were his long lost, finally recovered son. During my stay he often gave me a lift from my rented room to the laboratory. Most of what I know about genetics can be traced to those car rides. But the trip was not over when we arrived. Jack's office was at the far end of a

long corridor. Walking down the hallway he would stick his head into every lab and stop and talk with people on the way. He asked them about everything, the health of their kids, mother's broken leg, the weekend excursion, but first and foremost about the latest experiment. The people brightened visibly when they saw him and were always ready to stop for a chat or to ask him to come in and look into the microscope, at a bacterial plate or at a Drosophila progeny. Jack looked, listened, discussed, interpreted, proposed new experiments. Under his arm he carried his briefcase with all the papers he planned to finish during the day. Sometimes half a day passed before we arrived at his office where his secretary waited in despair!

I visited Jack's office 25 years later, long after his death. It has been refurnished as a conference room. It bears Jack's name. A silver plate on the wall reminds us of the unselfish inspiration he provided to everybody in his environment.

Jack succeeded in communicating the notion that biology is the most exciting science. He told me about whole worlds I had never heard about. Barbara McClintock's discovery of transposons in maize was one of them. Jack was one among the dozen or even fewer geneticists who understood what McClintock was talking about. He already knew, 10–15 years before most others, that her findings were going to revolutionize biology.

Jack's corridor was a wonderland for me at the age of 25. Briggs and King experimented with nuclear transplantation to enucleated frog's eggs. The question was whether the nuclei remained totipotent during differentiation. This was also relevant for cancer research. Could cancer cells contain a totipotent nucleus? The question was answered several decades later, by Beatrice Mintz at the same institute—at least answered so far as diploid teratoma cells were concerned. A cartoon appeared on the wall of the same corridor where Jack and I so often walked in the morning. Two mice were talking to each other. One of them said: "My father was a cancer, what does your father do for a living?"

The Mintz experiment is still unique in showing that at least some cancers can develop by epigenetic changes. The majority are no doubt due to changes at the DNA level, however.

My other important master at the ICR in Philadelphia was the mouse geneticist Theodore Hauschka. Through him I became acquainted with the inbred mouse. He had also taken a direct interest in my experiments. He gave me my own room in the perfectly organized mouse colony where I was in full operation for days on end. I compared the ability of different solid tumors to grow in the fluid form in the abdominal cavity. When they were reluctant to behave according to my wishes, I tried to select variants that would. At the same time I began to wonder whether the "histo-

compatibility genes" that were shown to govern the transplantability of tissues might provide me with the right system to substantiate our speculations on variation and selection within populations of tumor cells?

Despite my loneliness and separation from Eva, alleviated only somewhat by the letters I mailed her daily, I enjoyed being in America. In addition to the positive attitude of Schultz and Hauschka, the environment of the whole laboratory was highly supportive for a young man. There was a wholesome difference compared to European laboratories, particularly with regard to teacher-student relationships. It can best be summarized by a statement of the Danish biochemist, Lindeström-Lang: "The greatest accomplishment of the American revolution was to establish the right of young students to ask foolish questions."

During my stay in the United States I lost part of my emigrant complex. Hungarian emigrants in a comparable situation commented later that my initial shyness has turned into its opposite in the American setting. It may have seemed so. I was no longer afraid to ask questions, to inject myself into the conversation of learned professors, to speculate, and to risk making a fool of myself. I began to feel that this was not only my natural right but my responsibility.

Tumor Progression by Variation and Selection

The work on the conversion of solid into ascites tumors turned out to be quite interesting (2). Lymphomas and leukemias often converted immediately, while carcinomas and sarcomas refused to grow in the ascites form at first. Some of them could be converted gradually, however, by passaging the few desquamated, freely floating tumor cells in the peritoneal fluid. Using a simplified form of the Luria-Delbrück fluctuation analysis, I could show that this conversion was due to the selective enrichment of a small number of spontaneously occurring variants.

After four months in the United States, I returned to Sweden with 200 mice, anxiously guarded in my New York hotel room overnight and during the plane trip of more than 24 hours, to the great displeasure of my fellow passengers.

Back in Stockholm, the ascites tumor variants turned out to be stable. They retained the ability to grow in the peritoneal fluid immediately after inoculation, even after reconversion to the solid form and subcutaneous propagation over extended periods of time. The ascites adapted tumors were also more metastatic, less adhesive, and had a higher surface charge than their original nonadapted counterparts (3, 4). A comparison of our findings with Leslie Foulds' (5) work on tumor progression and Jacob Furth's studies (6) on the change of hormone dependent to autonomous tumors convinced us that we had hit an unusually well-defined case of

progression (7). It appeared to have a certain clinical relevance, at least at the conceptual level, because it showed that tumor cell populations were heterogeneous, and subpopulations could differ in their metastatic properties. But where did we go from here?

Tumor Immunology

To study variation and selection in tumor cell populations, it was obviously necessary to study variation first. We were looking for cellular markers, determined by known genes that could be detected at the cellular level. We found them in the recently discovered H-2 antigens of the mouse. George Snell has just started to distribute his first H-2 congenic mouse strains. We had induced tumors in H-2 heterozygous but otherwise congenic F_1 hybrids and isolated haplotype-loss variants by transplantation to the parental strains (8). Single haplotype-loss variants could be readily obtained, but in frequencies that varied widely between different tumors, even if they had been induced by the same agent and in the same host genotype. This biological variability was no longer a surprise to us, after the variations in ascites convertibility that we had encountered previously. Double H-2 haplotype losses were extremely rare.

Around this time, in the mid-1950s, a former colleague from medical school started to make extravagant claims concerning the prospects for the immunological prevention and cure of human cancer. He had immunized a horse with pooled tumor tissue and was firmly convinced that his serum reacted with a universal tumor antigen. He advocated immediate vaccination against cancer. The newspapers made a big splash. He was supported by some of the most powerful professors of microbiology and virology who had no experience in cancer. He injected himself with a HeLa cell derived "cancer vaccine" on TV. The public regarded him as a hero, particularly since the newspapers started to accuse the "cancer establishment" of lacking any concern for preventing cancer, due to vested interests.

The few of us who actually worked with cancer cells were profoundly sceptical. This could just not be true. But what was the real situation? Would tumors elicit immunity in their own inbred strain of origin?

My previous work with Hauschka left me imbued with a healthy scepticism against most earlier research in tumor immunology. The field was dominated by misinterpreted artefacts of experimentation with noninbred mice. The confusion between transplantation immunology and tumor immunology prevailed during the entire first part of the century. Only several decades after the development of the inbred mice by Little, Strong, Tyzzer, McDowell and others, and after the formulation of the "transplantation laws" by George Snell, was it gradually realized that the so-

called "transplantable tumors" violated histocompatibility barriers because serial homografting had selected them to outpace the rejection response. If the balance was tilted in favor of the host, e.g. by pre-immunization with attenuated tumor cells, it could reject the tumor. This easily won immunity could not be reproduced with tumors that had arisen in homozygous mice and were tested within their own strain. But what would happen at a more modest level of ambition? Could immunity protect the syngeneic host against near-threshold numbers of tumor cells? Clinicians and pathologists have always maintained that only a small proportion of disseminated tumor cells could grow into metastases in the human patient. Could an immune response that fell short of protecting the host against an established tumor still reject disseminated cells, in analogy with concomitant immunity in antiparasite responses?

Just as we started to think about these matters, Foley (9) and Prehn & Main (10) suggested that chemically induced mouse sarcomas, but not spontaneous mammary carcinomas, could elicit a state of immunity in syngeneic mice. The data were persuasive but still not fully convincing. Did chemically induced tumor cells really possess a distinct antigenicity of their own, or did these experiments merely reflect a residual heterozygosis in the inbred strains? It was obvious that the question could be decisively settled if it could be shown that the primary host could be immunized against its own tumor.

Using a combined scheme of tumor induction, operative removal, immunization with irradiated autologous tumor cells, and challenge with graded numbers of viable cells, we could show that methylcholanthrene-induced sarcoma cells were indeed capable of inducing true rejection reactions in the original host (11). Different tumors varied in their immunogenicity over a 5 log range of cell doses, required to break the state of immunity. Another and even more striking manifestation of biological individuality concerned the individual distinctness of the tumor antigens, also noted by Prehn, Baldwin, and Old (12–14). Each tumor could only immunize against itself. Cross-reactions were rare and irregular. The total number of possible specificities is still not known. We found no cross-reactions among more than 20 tumors. Hellström could not immunize mice against MC-carcinogenesis by using pools of a dozen tumors for immunization, while Old reported a certain preventive effect after the use of nonspecific immunomodulators that acted presumably by boosting the host's own responsiveness.

The nature of the carcinogen was not immaterial in determining the immunogenicity of the chemically induced tumors. Among the aromatic hydrocarbons, MC, BP, and DMBA induced sarcomas with decreasing immunogenicity, in that order. Sarcomas induced by the implantation of

cellophane film were hardly immunogenic at all (15). In the rat, Baldwin found that most azo dye–induced tumors were highly immunogenic, whereas acetylaminofluorene-induced tumors and spontaneous fibrosarcomas were not immunogenic at all (16).

Several decades have passed since these findings, but the nature of the TSTA (tumor specific transplantation antigen) of the chemically induced tumors is still a mystery.

Antigenicity of Virus-Induced Tumors

In 1958 I went to the Canadian Cancer Conference, in Honey Harbor, Ontario. Stewart and Eddy's pioneering work on the polyoma virus was still very new. Most participants were flabbergasted by the number and variety of the tumors that arose after the inoculation of the virus into newborn mice. Burnet was one of them. "Sir Mac" had recently shifted from virology to immunology and had developed a very negative view of the role of viruses in cancer in the course of this transition; he considered all virus-induced tumors as laboratory artefacts. Viruses were essentially cytopathic, and he saw no place for any true tumor inducing effect. Confronted with the polyoma story, he formulated immediately a new hypothesis. It was based on the only observation of Stewart and Eddy that turned out to be incorrect. They claimed that polyoma tumors were not transplantable. This was due to the accidental use of heterozygous mice, however.

Burnet suggested that polyoma virus may destroy some unknown, systematic "growth-controlling center," a possible "hypothalamus-like" homeostatic regulator of cell renewal in many different tissues. This would explain the ability of the virus to cause tumors in many different tissues. These tumors would not be transplantable to mice that have not been similarly conditioned by polyoma virus.

Hans-Olof Sjögren had just started to work with us at this time. Stimulated by Burnet's idea, I asked him to test the transplantability of polyoma tumors in unmanipulated and polyoma-infected syngeneic mice. The result was the exact opposite of what was predicted by Burnet's hypothesis: the tumors were readily transplantable to untreated mice, while small, graded numbers of cells were rejected by virus-inoculated syngeneic mice (17). The resistance of the virus-infected mice could be transferred adoptively with lymphocytes but not with serum. Both Karl Habel and our group later showed that antiviral immunity was neither necessary nor sufficient to induce rejection. Polyoma-induced tumors or transformed cells induced rejection, whether they released virus or not. All polyoma-induced tumors were rejected by the immunized mice, irrespective of tissue origin, but they did not reject tumors induced by other viruses or by chemical agents. We

have therefore developed the concept of a polyoma-specific transplantation antigen (TSTA) that was present in all tumors induced by polyoma, but not in tumors induced by other agents. We and others later found that similar group-specific rejection-inducing antigens were present on other virus-induced tumors (18). The retrovirus induced leukemias were particularly useful for the study of both humoral and cell-mediated reactions, as was shown by Old et al (19), and by our group.

Moloney virus-induced lymphomas were particularly useful for these studies, since they gave a brilliant membrane fluorescence reaction with the sera of preimmunized, syngeneic animals. Nevertheless, it was not possible to distinguish the rejection inducing antigen from the viral glycoprotein that accumulated on the surface. Different Moloney lymphomas induced in the same inbred strain differed in their rejection inducing potential. This system has permitted a distinction between immunogenicity and immunosensitivity, and we could show that they were independent variables. The former correlated with virus release, while the latter did not.

The Department of Tumor Biology

During the years of our transition from H-2 antigens to tumor immunology, our department developed rapidly. It was formally established in 1957, against all odds. Previously, both George and Eva had become assistant professors in Caspersson's Department of Cell Research (in 1951 and 1955, respectively), but our appointments were limited to a maximum of 6 years. Unless one acquired a tenured position, one was out of the research system. But no tenured positions were available in our field, which had not been previously represented at the Swedish universities. To circumvent the inflexibility of the university system, a number of "personal professorships" had been established for individual scientists, but some years before this time, the government decided to stop creating new positions of this type. Science was too expensive already for a country of eight million, they said; and it was also undesirable to continue the traditional recruitment of medical students into research. There was a shortage of doctors that made the authorities very sensitive about this, particularly since all higher education was financed by the taxpayers, and there was heavy competition for admission to the medical schools.

I had a good offer from the ICR in Philadelphia, and we seriously considered moving to the United States. Meanwhile, the Karolinska Institute, the Medical Research Council and the Swedish Cancer Society joined forces to initiate a parliamentry move, requesting the establishment of a Department of Tumor Biology with George Klein as its first head. This move was supported by representatives from the four major political

parties, but it failed to convince the Government. Decisions about budgetary matters rest with the Parliament, however. A parliamentary committee dealt with the matter on April 30, 1957. The odds were against us. The committee had 13 members of the ruling Labour Party and 12 members from the three major opposition parties. It was expected that the move would fail with a majority of at least one vote. In fact, the opposite happened. One Labour Party member (unknown to us) decided to vote with the opposition parties and the Department was established, as of July 1, 1957. Numerous medical and PhD students interested in research joined our group. With the support of the National Institutes of Health of the United States and the Swedish Cancer Society, the department expanded rapidly. The accumulation of married couples who pursued research together was a peculiar feature of the lab that has remained with us ever since. In the early years, the Hellströms, the Möllers, the Sjögrens, the Nordenskjölds, the Nadkarnis, and the Ozers were some of the examples. At one point we had seven married couples working at the lab at the same time, surely a world record.

The problem of space became overwhelming in the late 1950s. Again, there was no provision or precedent for the type of support that was needed. We were facing the possibility of having to return the first major NIH grant we had received under the Virus Cancer Program. I turned to the Swedish Cancer Society, although with little hope since the statutes of this essentially private organization explicitly discouraged the support of building facilities. But the Chairman, Professor Hilding Bergstrand, drove through a positive resolution against all odds. A new laboratory building was constructed in 1961. It houses the Department even today.

Burkitt's Lymphoma

Sometime in the mid-1960s, Eva suggested that we should use our experience on virus-induced murine lymphomas to examine a human lymphoma with a presumptive viral etiology. Could we detect group specific antibody responses that might be helpful in tracing a virus? Burkitt's lymphoma (BL) was the obvious choice. The recent description of the highly endemic occurrence of the African form which is climate-dependent strongly supported the idea of a possible viral etiology.

I wrote letters to numerous hospitals in Africa and to international organizations, explaining our project and asking for tumor, blood, and serum. I received some polite letters in reply, promises of material, and lovely stamps which made my son happy. But the material was not forthcoming at all, apart from an occasional shipment that arrived broken or infected. Then somebody—I have forgotten who—advised me to write to Peter Clifford, ENT surgeon at the Kenyatta National Hospital in Nairobi.

I got no letter and no stamps in reply, but the material started coming in a continuous flow. It arrived with chronometric precision on the single direct flight from Nairobi, late Tuesday afternoon. Large dry ice boxes carried hundreds of sera, and a special wet ice package contained fresh biopsy material. There was always a long list in Clifford's own handwriting with all the essential details and a brief "good luck" message.

We worked together with Peter over a period of more than 10 years. We have published 45 joint papers, the first in 1966 (20), the last in 1974 (21). We worked and published together for several years before we had a chance to meet in person. This taught us a new lesson. For collaborative studies, we tried to find a colleague who was motivated to study the problem and to collaborate with us, no matter where he or she resided. But we hasten to add that we have never encountered another clinical collaborator like Peter Clifford. He had a profound interest in BL, ever since he introduced chemotherapy in the treatment of the disease and became fascinated by the remarkably good regression in most of the patients. Their long-term survival eventually turned out to be complete cure in 15–20% of patients, including those who had only received incomplete chemotherapy. This was quite different from the effect of chemotherapy on other types of B-cell lymphomas. Clifford was convinced that the immunological response of the patient was decisive. If it was effective, even incomplete chemotherapy could induce total and long-lasting remission. If it was not, even more effective forms of chemotherapy were ultimately unsuccessful. Peter hoped that we would find evidence for an antitumor response in his patients.

We changed our working habits. Every Tuesday night was "Burkitt night." We made living cell suspensions from the fresh tumors, reacted them with the patient's own serum and other sera, and tried to read the tests immediately to obtain clues for the continued work. It was not difficult to motivate our personnel to work through the night every Tuesday.

Eventually, numerous other laboratories requested material, in the United States, England, and Japan, and some of them became engaged in collaborative projects. We could identify a membrane antigen (MA) that was expressed in some Burkitt lymphoma–derived cultures, but not in others (20). When I presented these data at an ACS Conference in Rye, New York, in 1967 (22), Werner Henle gave a talk in the same session. He reported his results, obtained with an immunofluorescence test on fixed BL cells that he and Gertrud Henle had recently developed, later known as the VCA (viral capsid antigen) test (23). They already knew that the reaction was due to structural antigens of a newly discovered herpes virus, first seen by Epstein, Barr, and Achong in the electron microscope. Henle

showed that it was antigenically distinct from previously known herpes viruses (24). We decided to call it EBV.

The Henles' VCA test and our MA test showed a certain concordance. The same lines appeared to react or failed to react in both tests. At the Rye meeting we agreed to collaborate. This initiated a highly productive association that has lasted for 20 years, terminated only by Werner Henle's death in 1987.

Already in the beginning of this work we obtained definite evidence that MA was encoded by EBV (25). It is now known as one of the viral envelope glycoproteins. It assembles within the membrane of the virus-producing cells, and after virus release, it can also attach to other cells in the same culture if they carry EBV-receptors. With Jondal, Yefenof, and Oldstone, we later identified the B-cell specific C3d (CR2) receptor as the attachment site of the viral glycoprotein (26, 27).

By 1970, it was clear that Epstein, the Henles, and ourselves had only seen the top of the iceberg when we looked at viral particles, VCA or MA. They only appear in virus-producing cell lines, and only in some of the cells. With Harald zur Hausen, we found in 1970, however, that more than 90% of the African BLs and all low differentiated or anaplastic nasopharyngeal carcinomas (NPC) contained multiple EBV-genomes per cell, no matter whether they produced virus or not (28). In 1973 I [GK] have found with Beverly Reedman that 100% of the cells in EBV-DNA positive BL biopsies and cell lines contained an EBV-encoded nuclear antigen, which we decided to call EBNA (29). Today we know that EBNA consists of a family of at least six different proteins (30).

Several important discoveries have been made by others in the meanwhile. The Henles, Pope et al, and Nilsson et al found that EBV could readily immortalize normal B cells in vitro (31–33). Departing from a serendipitous observation on a laboratory assistant, the Henles discovered (75) that EBV is the causative agent of infectious mononucleosis (IM). With Svedmyr, we could readily detect EBNA-positive cells in the peripheral blood of mononucleosis patients (34), and the Henles and George Miller found that the saliva of these patients contained transforming virus. Transformation was thus a natural property of the virus, not a laboratory artefact due to the accidental isolation of a defective strain, as our colleagues in the lytic herpes virus fields initially surmised. Miller & Epstein have also shown that EBV can cause lethal lymphoproliferative disease in immunologically naive marmoset and owl monkeys (35, 36).

Mononucleosis appeared as an acute rejection reaction of the "immunologically prepared" human host, selectively conditioned by a nearly symbiotic relationship with EBV over millions of years, against the virally transformed B cells. We found that the peripheral blood of the acute IM

patient contains activated killer cells that can lyse EBV-carrying and other target cells (37, 38). Moreover, autologous mixed lymphocyte cultures between EBV-transformed B-cell lines and T cells of the same normal donor generated a proliferative and cytotoxic response equally as strong as that of MHC-incompatible allogeneic MLC (39). Later, Rickinson, Moss and Pope showed that the autologous mixed cultures generated specific MHC class I–restricted CTLs by repeated stimulation (40). Eva's group, Sigurbjörg Torsteinsdottir, and Maria Grazia Masucci in particular, showed that the CTL response was heterogenous, directed against different target epitopes (41). The nature and specificity of the relevant targets have not been clearly defined yet in terms of the known virally encoded proteins, although current evidence by Moss et al and by Thorley-Lawson, respectively, indicates that both EBNA-2 and LMP epitopes may serve in this capacity (42, 43).

Since the work of Townsend et al (44, 45) has shown that MHC class I–associated peptides of processed endogenous or viral proteins can serve as immunogens and CTL targets, it would not be surprising if even more among the seven known growth transformation–associated EBV proteins could serve as CTL targets. A similar reasoning can be applied to the polyoma virus–induced TSTA, discussed above. The recent work of Dalianis et al in our laboratory suggests that all three polyoma encoded T-antigens can elicit rejection responses of the TSTA-type.

The hypothesis that T cell–mediated responses inhibit the proliferation of EBV-carrying B cells in healthy seropositives and in IM patients was reaffirmed when we found with David Purtilo (46) that most and perhaps all lymphoproliferative diseases that appear in congenitally or iatrogenically immunodefective patients, like children with the X-linked lymphoproliferative syndrome or organ transplant recipients, carry EBV-genomes. Hanto, Ho, and others have later shown that these initially polyclonal immunoblastic proliferations may progress to monoclonal lymphoma (47, 48).

While the tumorigenic potential of EBV was clearly established by these and related findings, its lifelong, innocuous latent presence in more than 80% of all human populations has also suggested that disease occurs only as an accident. Even mononucleosis appears as an "accident" of civilization. Modern hygienic conditions have apparently interfered with the normal, disease-free ecology of the virus-host relationship, with its predominant symptom-free early childhood infection.

The "accident" of the EBV-associated tumors has now been largely clarified for Burkitt's lymphoma, as described below, while the pathogenesis of nasopharyngeal carcinoma, the most regularly EBV-carrying human tumor, is still not understood.

Oncogene Activation by Chromosomal Translocation

By 1970, it was clear that some important element was missing from the BL scenario. EBV has clearly contributed to the genesis of the high endemic form of the disease, since 97% of the African BLs carried the viral genome, whereas non-BL lymphomas did not (49). Moreover, the prospective study of Geser and de The (reviewed in 50) showed that children with a high EBV-load are at a greater risk to develop BL than are their brothers and sisters with a low EBV-load, as indicated by the antibody titers.

Since the number of EBV-infected B cells represents only a minor fraction of the total B-cell population even in persons with a high EBV-load, the presence of the virus in the majority of the African BLs can only be interpreted to mean that an EBV-carrying B cell runs a greater risk of turning into a BL cell under the conditions prevailing in the "high BL belt" of Africa than does its EBV-negative counterpart. This is to say that EBV contributes to the etiology of the tumor. But this is still not a satisfactory explanation; some essential element is obviously missing. BLs differ from the true EBV-induced lymphoproliferative diseases like fatal mononucleosis or the immunoblastic lymphoproliferative diseases in organ transplant recipients, with regard to their cellular phenotype (51). The latter resemble the EBV-transformed B-cell lines of nonneoplastic origin (LCLs). LCLs are permanently growing immunoblasts that express a set of activation markers but not CALLA or BLA. BL cells, on the other hand, carry surface antigen and glycoprotein markers that resemble resting B-cells, rather than immunoblasts (52, 53). They express CALLA and BLA but no activation markers (unless they drift to a more LCL-like phenotype during prolonged cultivation). Recently, Gregory et al found normal B cells with a corresponding phenotype in tonsil germinal centers (54).

For the understanding of BL pathogenesis, it is also important to remember that approximately 3% of the African BLs, and 80% of the sporadic BLs that occur all over the world are EBV-negative. Among the recent, AIDS-associated BLS, the incidence of the EBV-carrying form is currently estimated as 40–50%.

The discovery of the "missing factor" in the "Burkitt equation" started when Manolov and Manolova reported in 1972 (55) that a 14q+ chromosomal marker was present in about 80% of the tumors. The Manolovs came from Sofia, Bulgaria, to work with us in 1970, at the time when the chromosome banding technique was discovered by Caspersson and Zech. I suggested that they apply the banding technique to the cytogenetically unexplored BL that kept coming in from Clifford every Tuesday in excellent condition. They agreed rather reluctantly since they had hoped to learn

some immunology. But their cytogenetic work soon picked up momentum, particularly after Albert Levan agreed to consult and guide them. When George Manolov showed me the extra band that he found attached to the distal part of the long arm of one chromosome 14 in a BL biopsy, I first suspected some trivial reason, perhaps a constitutional variation (isochromosome), and suggested that the Manolovs should take a look at the fibroblasts of the patient. So they did, but they found that the anomaly was totally restricted to the clonal tumor.

After the Manolovs returned to Bulgaria, we continued the work with Lore Zech. She soon showed that the "extra piece" was derived from chromosome 8; the 14q+ marker was thus a product of a reciprocal 8; 14 translocation (56). Several groups found subsequently that approximately 20% of the BLs that had no 14q+ marker carried one of two variant translocations instead (for review, see 57). Chromosome 8 broke at the same site (8q24) and entered into a reciprocal translocation either with the short arm of chromosome 2 or with chromosome 22. All BLs were found to carry one of the three translocations, no matter whether they were high endemic or sporadic, EBV-positive or negative. The same translocations were only exceptionally found in non-BL-lymphomas, although 14q+ markers are quite common; they usually arise by reciprocal translocations between chromosome 14 and some other chromosome, with 11 and 18 as the most frequent participants. But BL-type translocations were also found in the form of B cell–derived ALL that resembles Burkitt lymphoma cells phenotypically and is often called Burkitt leukemia.

Meanwhile, another, quite independent cytogenetic study, entirely confined to mouse tumor cells, was progressing in our laboratory. It started when the Hungarian-Rumanian pathologist, Francis Wiener joined our group in 1970. He is still one of our closest coworkers. Wiener became interested in the role of chromosome 15 trisomy in mouse T-cell leukemia, and he was also the main cytogeneticist in the somatic hybrid studies, together with Henry Harris, mentioned below. In the late 1970s Wiener examined a series of pristane oil–induced mouse plasmacytomas (MPCs); he was working together with a Japanese guest worker, Shinsuke Ohno, and in collaboration with Michael Potter's group at the NIH. Our 1979 *Cell* paper described the MPC-associated typical (12; 15) and variant (6; 15) translocations (58).

Mouse plasmacytomas are very different from Burkitt lymphomas. The only common denominator is that both originate from cells of the B-lymphocyte series. We never expected to find anything in common between the two. Therefore, the fact that two apparently unrelated research projects, carried out by different cytogeneticists, led to the discovery of a common pathogenetic mechanism, based on almost exactly homologous

chromosomal translocations, was one of the greatest and most pleasant surprises of my entire scientific career. It was even more surprising that the highly speculative working hypothesis, formulated to explain the mechanism whereby the translocations contribute to the tumorigenic process in such a decisive fashion, turned out to be essentially correct.

The hypothesis was built on the fact that the recipient murine chromosomes of the dislocated fragment from chromosome 15 were known to carry the IgH (chromosome 12) and the kappa (chromosome 6) gene, respectively. Likewise, human chromosome 14 was known to carry the IgH cluster. We have therefore speculated that a proto-oncogene and probably *the same* proto-oncogene could be localized at the breakpoint of the murine chr 15 and the human chr 8. Accidental translocation of the putative gene to one of the immunoglobulin loci might have led to the constitutional activation of the gene, in analogy with the retroviral activation of the c-*myc* gene by the insertion of an ALV-derived LTR in the chicken bursal lymphoma, as described by Hayward et al.

I started to expose the hypothesis to the test of peer criticism in 1979. An outstanding molecular biologist, a good friend of mine, called it the "most hair-raising extrapolation from the centimorgans to the kilobases." It was. Still, the hypothesis was published in *Nature* in 1981 (59), but I was not fully convinced of it myself, until the critical moment during the summer of 1981, when I was waiting for a plane at Washington airport to take me to Tokyo. The waiting hall was full of people, mostly Japanese. There were only two telephones on the other side of the security check. They were busy most of the time. The plane was called up. Finally, one of the telephones was free. I tried to get hold of Philip Leder at the NIH. I wanted to hear whether he knew anything about the chromosomal location of the immunoglobulin light chain genes in humans. Leder came to the telephone. No, he hadn't heard anything; it was still unknown. But one of his colleagues had just come back from the recently held Human Chromosome Mapping meeting in Oslo. If I waited, he would try to ask if the colleague had heard anything.

"Final call." The last Japanese walked aboard, and I had to leave. At the moment when I was about to hang up the phone, Leder's voice came back: Yes, there were two small reports in Oslo. An English group had found that kappa is on chromosome 2. An American group had proved that lambda is on chromosome 22.

I ran on board. It was an intoxicating feeling! I knew for certain that the hypothesis was correct.

The molecular confirmation and clarification came in a virtual avalanche during 1982. Taking off from quite different points, Jerry Adams with Susan Cory in Australia and Kenneth Marcu in New York showed for

MPC, and Carlo Croce and Phil Leder for BL, that the translocations resulted in the juxtaposition of donor chromosome derived sequences and immunoglobulin gene sequences. Michael Cole's group has identified the transposed gene as c-*myc* (for review, see 60).

The subsequent development has led to many new insights, but it has also created some puzzles and paradoxes with regard to *myc*-regulation, constitutive activation, and certain details of the timing and regulation of Ig-gene rearrangement (for review see 65). With Francis Wiener and Janos Sümegi, we have also found a third Ig/*myc* translocation system (61–63), the spontaneous immunocytoma of the Louvain rat (RIC), developed by Hervé Bazin. A comparison of the translocations in MPC, RIC, and BL at the molecular level reveals more similarities than differences. In fact, it would be hard to find a comparable situation in cancer biology where three pathogenetically different tumors that arise from the same cell lineage in three different species show a similarly close pathogenetic mechanism at the molecular level.

The causal, i.e. rate limiting, involvement of constitutive *myc* activation in the genesis of the three tumors was deduced from the regularity of the Ig/*myc* juxtaposition that extended to cryptic translocations and complex rearrangements, where two or three successive genetic events had occurred (61, 64). Further confirmation came from recent facsimile experiments. Michael Potter and Francis Wiener showed (66) that introduction of an activated *myc* gene within a retroviral (J3) construct into pristane oil–treated Balb/c mice induced plasmacytomas that did not carry any translocations, provided they expressed the inserted (v-*myc*) gene. Meanwhile, Adams & Cory's group generated transgenic mice that carried the *myc*-gene coupled to the IgH enhancer (67). The mice developed more than 90% pre-B- or B-cell-derived lymphomas. Using the Australian transgenic mice, Francis Wiener recently found that Abelson virus infection, already known to increase the incidence and shorten the latency period of pristane oil–induced mouse plasmacytoma, has led to the appearance of plasmacytomas in the Emu-*myc* transgenic mice. The virus has obviated the pristane requirement and lifted the genetic restrictions to MPC susceptibility. These plasmacytomas were also translocation free.

That introduction of an activated *myc* construct was tumorigenic for B cells and obviated the need for the translocations could be only interpreted to mean that the naturally occurring constitutive activation of *myc* by the Ig-translocations provided an essential, rate-limiting step within the carcinogenic process. But it is not the only step. All tumors were monoclonal, even in the transgenic mice where *myc* was activated in all B and pre-B cells. Sequential activation of several oncogenes or, alternatively, loss of suppressor genes may provide additional steps. Feedback inhibition

by the clone that happens to get the upper hand first would be another alternative.

The Burkitt lymphoma story has also developed further in the meanwhile and has posed some new fascinating questions. We have suggested, for both conceptual and factual reasons, that the BL progenitor is a long-lived B-memory cell. In this scenario, antigenically stimulated B-cell clones that have previously expanded as immunoblasts, were in the process of switching their phenotype to CALLA- and BLA-positive, activation marker negative memory cells when, upon the waning of the antigenic stimulus, the translocation accident occurred. Due to the linking of *myc* to a constitutively active Ig-locus, the cells were unable to leave the cycling compartment, however. It could be shown that the translocation carrying "suspended resting cell" had several additional phenotypic properties that could facilitate its evasion from immunological control. Certain MHC class I polymorphic specificities were down-regulated in the BL cells, compared to EBV-transformed B-cell lines of normal origin. The BL cells also failed to express certain adhesion molecules present on the LCLs or expressed them at a low level. Even the EBV-encoded, growth-transformation associated nuclear and membrane antigens were down-regulated in the BL cells, with the exception of EBNA-1. This was paralleled by a relative resistance of the BL cell to CTL-mediated lysis (56).

It thus appears that the *myc*/Ig translocation promotes the malignant growth of the BL cell by several mechanisms. This may explain the extraordinary regularity of its presence in all typical BLs so far studied.

Tumor Suppressor Genes

I have recently reviewed this field in some detail (68) and concluded that we are probably approaching an era when the study of genes that can antagonize tumorigenic behavior will be equally as, if not more rewarding than, the study of the oncogenes. Our own commitment to this field started with a decade of another long distance collaboration, initiated by Henry Harris in 1969 (69). We have inoculated a large number of somatic cell hybrids, derived from the fusion of high malignant with normal or with low malignant cells, into genetically compatible and/or immunosuppressed mice. The hybrids were generated by Harris, and Wiener examined their chromosomes. These studies have firmly established the notion that tumorigenicity is suppressed by fusion with normal cells. It reappears after some critically important chromosomes, contributed by the normal cell, have been lost. Others have extended this work to human/human hybrids more recently and obtained similar results. Chromosomes that carry tumor suppressor genes have been identified by Stanbridge, Klinger, Sager and their associates (70–72). The field is now moving towards a more reduc-

tionistic analysis where microcell hybrids are taking the place of whole cell hybridization and c-DNA transfections are initiated to identify the suppressor genes and their products. Meanwhile, evidence for tumor antagonizing genes has also emerged from the study of revertants and particularly from the rapidly moving field of "recessive cancer genes" that contribute to tumorigenesis by their loss (73, 74). It is not clear if or to what extent there is a relationship between the genes identified by these three approaches.

Whither Tumor Immunology?

It is often asked if or to what extent the spectacular development of the oncogene field during the last decade may provide some new handles for targeting the antitumor response. The answer may differ in relation to oncogenes activated by regulatory or by structural changes, respectively. Up-regulation of a structurally normal oncoprotein is less likely to provide a rejection target than oncoproteins activated by structural changes, e.g. the products of the *ras*-mutations or the truncated growth factor receptors, exemplified by the tumorigenic variants of erbB or fms. Following Townsend's discovery that intracellular, endogenous proteins can be processed to peptides that combine with class I or class II molecules and can then serve as immunogens and/or as CTL targets, the structurally changed oncoproteins deserve serious consideration. Progress will depend on the expression of mutation-activated (compared to normal) oncogenes in non-immunogenic tumor cells—of which there are many—followed by the assessment of their immunogenicity and rejectability in syngeneic hosts.

Epilogue

As each of us is moving towards the approaching darkness, the sun is never setting over the vast oceans of science. It has been a rare privilege to live and work through the times when the genetic material turned from protein to DNA, when adaptive changes in cell populations—including antibody production—were unmasked as Darwinian variations and selection, when GOD became the rearrangement of immunoglobulin genes, violating the dogma that all somatic cells have the same DNA. Another central dogma was abolished when the RNA tumor viruses became DNA proviruses. Following closely in the wake of this discovery, the enthusiastic retrovirologists, searching for the universal cause of cancer, permitted the great cuckoo egg, the oncogenes, to hatch—almost imperceptibly at first, but with a rapidly increasing crescendo, towards the triumphant emphasis on the regulatory genes of the cells and their dysfunction as the key factor in the oncogenic process. Departing from even greater obscurity, the MHC system, once the esoteric pet of a few mouse geneticists, now occupies a

central place in virtually every area of immunology. It was a great time, and it still is, but it is only the stumbling, stuttering, premature foreshadowing of what lies ahead. We have barely scratched the surface.

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