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The strange road to the tumor-specific transplantation antigens (TSTAs)

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One of the great surprises of my first stay in the US during the fall of 1950 concerned the so-called "transplantable tumors" and their "immunology". During the first part of the 20th century, cancer researchers spoke about transplantable and non-transplantable tumors. The mice and rats were not inbred and transplantability therefore meant transgression of histocompatibility barriers, but most researchers were unaware of this. It was relatively easy to immunize against transplantable tumors and "tumor immunology" was an "optimistic" field due to this artifact. Theodore Hauschka, my first American mentor, told me about inbred mice and the laws of transplantation. Virtually all tumors were transplantable within their inbred strain of origin, but only some exceptional tumors were "transplantable" to foreign strains. The easy immunization and rejection experiments did not work when tumors were grown and tested in their own inbred strain of origin.

All of us who learned this lesson and worked with inbred mice - still a minority around 1955 or thereabouts - became convinced that tumor immunology did not exist. The immune system regarded tumor cells as "self" in the primary host and there was nothing more to it.

In spite of this clear, definite evidence that was available already in the early 50s, the artifactual, allograft-based "tumor immunology" continued to flourish during at least one more decade. Thousands of meaningless experiments were performed and hundreds of papers were published.

The "Tumor Immunology" session of the International Cancer Congress in London in 1958 was chaired by Peter Gorer. Together with George Snell, Peter pioneered the study of the H-2 system (the name was coined by him) and he would no doubt have shared the Nobel Prize with George Snell in 1980, had he not died earlier. The session in London took place in a lecture room at the City Hall, near the Royal Festival Hall. It was uncomfortably hot, there was no air-conditioning, and the windows could not be kept open because of the noise of the traffic outside. Peter was sweating profusely like all of us, and he had a little pile of headache pills and a glass of water in front of him. He was calling one speaker after another and listening to their 10-minute talks. Most of them showed "highly successful" immunization against the "transplantable tumors" and expressed great hopes about cancer vaccination. None of them had heard about the great advances in the genetics of transplantability during the 30s and 40s. Peter did not comment although (or because) he knew it was all wrong. After the last paper he made his first statement during the session, apart from the usual chairman formalities: "Open the windows! We need some fresh air".

Meanwhile, other things started happening. Ludwik Gross (yes, the same person who discovered the Gross leukemia virus, but that is another story) performed some not too well controlled experiments suggesting that chemically induced mouse sarcomas could be immunogenic in syngeneic mice. Subsequently, Prehn and Main confirmed this in critically controlled experiments in 1957 and showed that methylcholanthrene-induced sarcomas, but not spontaneous mammary carcinomas, could induce relatively weak but regularly repeatable rejection reactions. Their data also indicated that the chemically induced tumors did not cross react with each other in rejection tests and that each tumor was thus individually distinct by the rejection criterion.

We were still die-hard skeptics at this time. We suspected - wrongly - that even the experiments of Prehn and Main may have been flawed. Obviously, the ultimate evidence had to be based on experiments with the primary, autochthonous tumor host. We did these rather laborious experiments and published them in 1960 in Cancer Research (1). Yes, it was all true.

Spontaneous tumors - defined as tumors that have arisen without any experimental interference - were not immunogenic in our hands either. But how about the virus-induced tumors that were being reported at a rapidly accelerating rate, after the discovery of the Gross leukemia virus was confirmed by Jacob Furth, and after the polyoma virus was isolated by Sarah Stewart and Bernice Eddy? The dominating opinion swung like a pendulum, from a quasi-total lack of interest in viruses and cancer to the equally unjustified belief that perhaps all tumors were due to viruses.

In 1958, I was going to the Canadian Cancer Conference in Honey Harbor, Ontario. One of my earlier guest workers, Arthur Axelrad from Toronto, was meeting me at the airport. Six of us squeezed into his normal American-sized car. I sat on the front seat, in the middle. On my right, there was Sir Macfarlane Burnet, probably the most influential medical biologist in the world at the time and during the preceding decades. He was equally well known for his profoundly analytical reasoning about some of the most important medical and biological problems as for his categorical and opinionated generalizations that often turned out to be wrong. Before that happened, however, he usually irritated people into doing a large amount of work just to disprove him. By the time Burnet's idea was definitely disproved, a new science had grown up, over and over again.

Three ladies sat on the back seat. Lady Burnet, on the left, was extremely worried about Arthur's fast driving. This was not unusual since she often worried about Sir Mac's health, as was also the case two years later when he shared the Nobel Prize with Sir Peter Medawar for having formulated the concept of immunological tolerance. On that occasion Lady Burnet was continuously concerned about her husband's ability to go through all the festivities. But that was in the future. At this point, she tried to make some polite understatements about the dangers of fast driving, but Arthur was very anxious to catch what Burnet was saying to me (about immunological tolerance) and did not perceive this at all. The wind was blowing through the open windows and it was hard to hear from the back seat what was said in the front. This was the reason that the two other ladies, Sarah Stewart and Bernice Eddy, the recent discoverers of polyoma virus, were even more anxious to hear our conversation. They suspected, wrongly, that it might relate to the topic of viruses and cancer. They knew that Burnet had recently dismissed the whole notion. He was quoted to have said that all viruses are cytopathic, and all talk about viruses causing cancer was therefore nonsense. As it turned out later, Burnet had not yet heard about polyoma virus at the time when we were sitting in Arthur's car. He was to learn about it at the meeting in Honey Harbor. He did not have the faintest idea who the two rather strange-looking ladies were on the back seat, nor did he show any interest to find it out.

Finally, Sarah could not stand the suspense any longer. She popped her head between Burnet and me and said: "Excuse me, Dr Burnet, but what do you think of viruses and cancer?"

Burnet turned slowly, like a gun taking aim. In a few well-chosen words he told her that he did not believe there was anything in it at all.

Stewart and Eddy were shattered. They had no doubt that the remark was directed against their work. They sank back, annihilated, and the back seat remained immersed in stony silence during the rest of the trip. Neither Axelrad, nor Burnet noticed anything, while Lady Burnet who did sense the change could not imagine what it was due to.

At Honey Harbor, Stewart and Eddy presented their spectacular results. Yes, the new virus, called polyoma ("many tumors") did induce a large variety of tumors, almost the entire pathology textbook, except leukemia, from which it was isolated in the first place. The comment in the coffee break was: "They must have a hole in their filter or a hole in their heads". But, unlike the case of the Gross virus where confirmation took 5 years, the polyoma work was rapidly confirmed by others.

How did Burnet respond to the data presented at the meeting? He could not doubt the well documented evidence, but still would not believe that viruses could transform normal cells into tumor cells and would then persist in the tumor without cytopathic changes. He therefore postulated, in a typical Burnetian impulse, that the virus acted by "knocking out" some unknown central growth controlling organ that kept normal tissues within their confines. Due to the loss of the postulated growth controls, tumors would bloom up everywhere.

Burnet's speculations that were thinner than air, together with my conversations with Stewart and Eddy, gave me a new and totally wrong idea that led to an unexpected result.

Everything Sarah told me about polyoma was correct, except one small but important detail. This was the only wrong information I ever heard Sarah say. She told me that polyoma-induced tumors were not transplantable. I did not realize that Sarah was quite ignorant of mouse genetics, in spite of the highly sophisticated mouse tumor

pathology that was part of her expertise. She performed her few transplantation experiments with polyoma tumors on non-inbred Swiss mice.

I had a brainwave. It was triggered by the combination of Sarah's flawed transplantation experiments and Burnet's groundless hypothesis. If polyoma tumors were not transplantable, perhaps Burnet was right and polyoma tumors were not autonomous unless the "central growth controlling organ" was knocked out by virus infection. On homecoming, I suggested to a new student - no other than Hans Olof Sjögren - that he might test one aspect of the hypothesis experimentally. My line of reasoning was as follows: Let us induce polyoma tumors in one of our inbred strains. We shall then take two groups of the same strain. One will be infected with polyoma virus, so as to "knock out" the hypothetical growth controlling center, while the other will be left untreated. If Burnet is right, the tumor should be transplantable to the virus treated, but not to the control mice.

It was not an easy experiment to do. We could not introduce the highly contagious polyoma virus into our mouse colony. Thanks to the senior professor of pathology Nils Ringertz, we could set up a separate, totally isolated little mouse colony at his institute at Sabbatsberg Hospital.

Sjögren's experiments turned out to be highly informative, but gave the opposite result from what was expected on the basis of Burnet's speculation. The tumors were perfectly transplantable to untreated syngeneic mice, proving Sarah wrong, but not or only to a very limited extent to the polyoma-infected mice (2). Together with his sister Ingegerd who by this time has become Ingegerd Hellström, Sjögren showed in what became his thesis that polyoma-induced tumor cells carried powerful TSTAs, in contrast to the individually distinct methylcholanthrene-induced tumors. However, in immunization-rejection tests, all polyoma tumors cross reacted with each other, irrespectively of their histology. They did not cross react with non-polyoma tumors, even if they were of the same histological type. Similarly, group-specific rejection-inducing antigens were subsequently found by our group, as well as by other groups, in many other virus-induced tumors. This also became the basis for my wife Eva's and my choice of Burkitt's lymphoma as the first human tumor we wanted to examine in a similar way, around 1965, but that is another story (3).

The polyoma-TSTA work was developed much further by Sjögren, both before and after his move to Lund University as professor of tumor immunology and later by Tina Dalianis and her co-workers. There is one more point in that context that needs to be made about the dynamics of conceptions and misconceptions.

Both our group and Karl Habel's at NIH, who detected the same polyoma-TSTA on virus-induced hamster tumors, believed that the antiviral response was distinct from the anti-TSTA response and that therefore TSTA could not be a viral antigen. Personally, I was thoroughly convinced that the TSTA is virally encoded, because only that could explain the cross reactivity of tumors induced by a single virus in many different tissues and in different species. The alternative suggestion, virally-induced cellular, e.g. fetal, antigens did not appeal to me at all. But I did not believe that the polyoma T-antigens could be responsible, because the anti-T antibody response and the anti-TSTA (rejection) response could readily be dissociated by various experimental manipulations. We thought that we had excluded polyoma large T particularly well, which we considered unlikely from the beginning, because it was a nuclear antigen. This was well before Townsend's discovery of antigen processing and peptide association with MHC class I antigens, and even before Zinkernagel and Dougherty's demonstration that cytotoxic T cells recognized MHC class I associated peptides on the cell surface.

Of course, we were quite wrong. TSTAs are exactly that: processed peptides, derived from the transforming viral proteins, associated with appropriate MHC class I molecules.

The TSTAs of the chemically induced tumors are still a mystery.

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