Remembrances of Hilary Koprowski from a Surgeon's Perspective

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HILARY KOPROWSKI WAS ONE OF THE MEDICAL GIANTS that inspired my life and career—therefore the larger than life gratitude and affection I feel for him. Although this reality may color the remarks that follow, they are offered as my best personal recollections.

I was introduced to Dr. Koprowski in the late 1970s by Dr. Michael Mastrangelo, an oncologist with a primary interest in melanoma at the Fox Chase Cancer Center, not long after I relocated there from the Surgery Branch of the National Cancer Institute. I had been working at the NCI as a senior investigator, primarily focusing on questions related to cryopreserving human lymphocytes as indicators of post-operative tumor status after immunotherapy or removal of tumors.

Looking back, it is hardly believable how little I knew about lymphocytes at that time. These were the days when “tumor-specific antigens” as identified by polyclonal antisera were only beginning to be talked about, and considerable efforts were being made towards immunotherapy of melanomas at the NCI Surgery Branch. During this time, I had the opportunity to learn some of which I should have known earlier. Mike Mastrangelo and I had common interests, and I was still collecting sera from all manner of consenting cancer patients in the hopes that we might identify some indicator of a specific immune tumor response. Mike had heard about the amazing properties of a new creation, then called “somatic cell fusion products,” which were the monoclonal antibodies produced at The Wistar Institute under Hilary Koprowski's direction. He then made the introduction that was to be transformative for me.^(1–3)

Hilary Koprowski was a master at transferring enthusiasm. He knew how to communicate to those less informed, such as myself, the potential of the new technology and its revolutionary use in identifying and defining complex macromolecules. This approach was viable not just for those mediators of human biology and my interest in malignant disease, but for countless other uses as well. Hilary was well acquainted with the concerns of clinical surgeons who cared for cancer patients and the need for alternative therapeutic modalities that could be added to local or regional approaches to human cancers. Chemotherapy was at that time not the answer for many. Koprowski rightfully recognized the potential for these exquisitely specific reagents to identify and treat distant metastases that were undermining local therapeutic modalities.

I do not believe that he coined the term “magic bullet” that was pinned on monoclonal antibodies in those years, but he shared the desire to give these reagents the broadest evaluation for their potential benefit in the therapeutic armamentarium.

A steep learning curve was facilitated by many investigators at The Wistar Institute, and I became convinced that monoclonal antibodies were the answer to the shortcomings of the antisera we previously used to identify tumor-specific antigens, which could now be identified as individual molecules. Those scientific investigators, including Dr. Zenon Steplewski and Drs. Meenhard and Dorothy Herlyn, also introduced me to a level of scientific investigation that was greater in magnitude from what I had previously experienced. They taught me that there were fundamental differences between a scientific investigator and a clinical one, a combination that made, at that time, a fruitful partnership. Many laboratory doctors expected human patients with malignant disease to react to interventions as would an inbred strain of rodents. They also did not appreciate the variability of what was included under one label of a specific human disease, for example, “breast cancer.” The fact that all adenocarcinomas of the breast were not the same, as far as its behavior in a human was concerned, was new information to many. So we each occupied a position of ignorance, and we found the partnership compensated for this to some degree.

During this time, a great number of monoclonal antibodies (MAbs) against a variety of human and viral tissues were generated and characterized at The Wistar Institute. Numerous investigators were using these reagents to define many mediators of metabolic processes involved in those human conditions that could be studied *in vitro*. The most interesting of these antibodies for the clinical surgeon were a few that had been created from a human gastrointestinal track adenocarcinoma. Drs. Steplewski, Herlyn, and Herlyn had made extensive evaluations of monoclonal antibodies raised in response to a human colonic adenocarcinoma. They also had demonstrated *in vitro* killing of these human tumors in cultures and in a nude mouse system.^(3,4–6)

The MAbs that had shown specific responses when tested *in vitro* were used as potential tumor markers in very preliminary studies. The carcinoma embryonic antigen (CEA) had been used in clinical medicine for about a decade, but it had many of the failings that these new reagents hopefully

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would overcome; therefore the CA19-9 antigen, a monosialoganglioside associated with gastrointestinal tumors, had a higher specificity.^(7,8) Because of this team's preliminary work with two of these antibodies, one with cytotoxic activity, another with binding characteristics to sera from tumor-bearing animals, there was considerable enthusiasm to expose these reagents to trials on human material.

Evaluation of Monoclonal Antibody as a Clinical Marker

Studies on patient sera to evaluate the potential for one of the antibodies as a diagnostic marker (CA19-9) for a circulating specific shed molecule from the tumor was relatively easily accomplished because the Fox Chase Cancer Center already had a sera bank in place and a program for storing pre- and postoperative samples from patients with primary cancers. A significant population of patients with non-malignant disease were also gathered and used as control samples. These samples were collected during the patient's return visits over a multi-year timetable and, as such, a few recurrences were unfortunately expected.

A protocol was written for the selection, handling, and transport of samples and controls and the mechanism by which to have the sera assayed by a blinded investigator. Patient clinical information was collated with assay results by a third individual who did not know the patient nor his or her clinical status. When these data were combined and reported to this investigator, it led to some interesting and exciting phone calls, because of some of the surprise matches to subsequent clinical findings (known by then to the clinicians). There were also results that did not coincide with what we expected, and the statisticians did the right thing by including them as reported.

These data were convincing enough, though hardly perfect, to support more rigorous studies that would allow direct comparison to CEA, the marker of the day. It also led to the initiation of an intellectual property process. This was entirely new ground for me; the entire process of presenting the subsequent results to the FDA was clearly over my head, and the FDA was never as impressed with the data as we were. This was an education as to the workings of the agency. Even so, the data did find its way into the academic literature.

Antibody Binding Into Human Tumors

In vitro studies

Multiple *in vitro* studies had shown specific binding of the monoclonal antibody called 17-1A to cultured human colonic tumor cells, but not to normal human cells, as well as tumor necrosis in nude mouse models.^(4,6) We next embarked on an experiment designed to determine if we could show specific binding of this antibody in a cancer-containing segment of a human colon shortly after it had been surgically removed from a patient. We accomplished this with a perfusion device that delivered a physiologic perfusate containing the antibody through the mesenteric artery. Control antisera to non-colonic antigens were used as well as monoclonal antibodies that bound to antigens that were shed from colonic tumors. The results of ten trials of specimens ranging over many segments of the large bowel and one that contained a portion of the small bowel led to the conclusion that there was definite binding to the mucosal tumor cells as well as detectable

binding to other tissues, mucosa, or cells in the specimen.⁽¹¹⁻¹³⁾ Antibody binding to small bowel mucosa was also detected in the one specimen that contained it. Although some tumors did not bind antibody and normal mucosa did in some preparations, some specificity was noted in some of the tumors, suggesting the need for further study.

In vivo studies

As evidence for the capacity of these new monoclonal antibody reagents to bind to discrete macromolecules of cellular components and differentiate malignant from normal cells became available, there was increased pressure to explore whether they would bind specifically and solely to the cancer in humans and not to normal cells. The potential for both diagnostic and therapeutic strategies were believed to be limitless. Enthusiasm was further increased when it was demonstrated that these reagents could be fashioned to be carriers for not only potent cell metabolic poisons such as ricin, but also isotopes capable of being used for detection of and potentially destruction of malignant tissue in patients.

The timing of this information coincided with the development of more sophisticated human experimentation control committees in hospitals that were doing clinical trials using patients. Most of the people on these committees had not heard of monoclonal antibodies at this time, and had no experience demonstrating the potential with the infusion of such a substance into a human. Truth be told, neither did some of the investigators, even while every endeavor to understand and anticipate risks were employed.

Research from The Wistar Institute and other centers, cancer or otherwise academic, on the potential utility of these reagents, including evidence of tumor destruction in animal studies,^(4,6,14,15) was growing exponentially, but studies addressing potential problems that might occur in human patients did not exist. There were not even suggestions beyond the obvious that these reagents were of mouse, not human, origin. The effects of antisera from foreign species were well known, but what portion of this knowledge translated to the murine monoclonal antibody as a purified entity was new territory. These subjects were discussed, and cautions and procedures to deal with potential untoward events were recommended or demanded; after long meetings and debate, permission was granted for, at first, a very limited trial using a monoclonal antibody attached to a radioisotope that could be detected by conventional scanning devices of the day. The issue of the isotope chosen was easy, as the formulation to be employed thankfully could be made so that the antibody would still bind to its target while also attached to an isotope used in routine clinical studies. The isotope did not seem to impair binding to tumor targets in any noticeable manner.

The process then became one of patient selection based on the likelihood of sufficient tumor being present in the scanned areas and likelihood of sufficient binding in time frames that were completely unknown. This implied identifying a patient with a colon carcinoma who also had a significant chance of metastasis, clinically not identifiable by current screening techniques, yet if detected would alter the patient's treatment plan. With Human Experimentation Committee approval, the antibody-isotope was administered.⁽¹⁶⁾

This experience fueled the debate about the possibility of significantly broadening the scope and goals of human

clinical trials. Since no adverse reaction to the murine part of the infused reagent occurred, pressure to start the process of designing and carrying out therapeutic trials of monoclonal antibodies with proven cytotoxic properties in animal studies mounted significantly. There had been no untoward allergic reaction to a single infusion of the monoclonal antibody, and a phase I human study of the antibody, not attached to any isotope and in gradually increasing doses, was designed and debated at length in Human Experimentation Committee meetings. Patient selection was obviously critical, and the procedures for this were discussed at length, even after approval was granted. There were no shortage of patient volunteers, but suitable candidates that completely fit the study design criteria were not as common as might be expected, though not scarce.

The design of the trial was to evaluate safety, with tumor response evaluation a secondary issue, but obviously one of great interest. It must be remembered that the imaging technology in the 1980s was much more limited than that of present day. Patients with observed metastasis during an operative procedure had lesions that were not measurable short of operative intervention. As is true today, the light microscopy of an individual tumor does not necessarily correspond to the rapidity of its growth. Therefore there were naturally many impressions as to events that were observed that were not and could not be based on hard evidence. That a single infusion of a purified monoclonal antibody, admittedly a foreign globulin, caused no untoward reaction was no surprise, and as the dosage administered increased, the chances that any of the trial participants had previously been exposed to murine proteins was extremely small.

As occasionally happens in trials of new therapeutic modalities in human medicine, an event in the very early stages of a trial can have extraordinary consequences. This happened in a trial using a monoclonal antibody designed to destroy a human lymphoma. An implied response that was much better than expected happened in the early part of the trial, leading to great expectations that were never again observed. A similar event happened in our trial.

The clinical evolution of colon carcinoma, even when metastatic, is very variable, and can take a considerable period to become manifest. Many of our and other sera studies of circulating antigens confirm this. So when soft signs of improvement were encountered, they carried more weight of a good outcome than actually occurred.

Nonetheless, pancreatic cancer's progression is considerably fast and it is easier to make judgments about its clinical progression. In the early part of our trial, we studied a man who had been found to have metastatic pancreatic cancer as a consequence of having a cholecystectomy for gallbladder-related symptoms and a diagnosis of cholelithiasis. Hepatic metastasis was confirmed during the operation and subsequently on hepatic scans. Pulmonary metastasis was also suspected by lung radiographic studies. The patient was accepted into our trial and was infused with the 17-1A antibody. Over the following months, then years, he remained asymptomatic, and repeated diagnostic studies seemed to indicate resolution of images initially interpreted to be metastases.⁽¹⁷⁻²³⁾

Understandably, this was viewed as remarkable and in some quarters unbelievable. Pathology slides and radiographic material were constantly being sent hither and yon,

even to the most senior offices of the NCI. Consequently, this material was not always immediately available for local visitors and caused many an uncomfortable moment, such as when the press descended on us and The Wistar Institute soon after President Reagan was found to have colon cancer. Because of our monoclonal antibody trial, we were in the news, and we had a hard time producing x-rays that were being photographed away from the hospital. This seemed to be of little concern to the producer of the news show; the next day the x-ray was displayed even though it took a week before it was back in the hospital.

Because of this and the seemingly good symptomatic response of many of the patients in the early part of the trial, I was invited to accompany Dr. Koprowski to many meetings that included the most prestigious investigators in the various fields involved both in the United States and abroad. I had the opportunity to drive in Germany with two investigators, RNA virologists, whose work was supported by the 1970s War On Cancer, but who made their most significant contributions to understanding the HIV virus a decade later.

I had the opportunity to meet foreign investigators who were collaborating with Dr. Koprowski in their labs and hospitals, as well as when they visited the United States. Many of these collaborators continued the lines of research that these early studies using a murine antibody suggested. Although we had no problems with an untoward reaction to the foreign antigen, any wheeze in any patient caused a bit of anxiety to those standing by. Thankfully no side effects emerged in the people in our trial because repeat exposure was not considered, and there was good evidence that some patients definitely had recognized the murine antigen as foreign.

After the initial infusion trial of increasing doses of the 17-1A murine monoclonal antibody, there was recognition that it was not a magic bullet for pancreatic adenocarcinoma. Results from many patients diagnosed with colon adenocarcinoma were not dramatic, and humanized antibodies were becoming available. As new avenues began to be explored, personal family requirements prevented me from actively taking part in additional studies.

Despite my separation from these later studies, my fondness for Dr. Koprowski and my extreme interest in all studies that The Wistar Labs conducted for patients with cancer persisted. I became a cheering bystander and remained a devoted life-long friend.

Author Disclosure Statement

The author has no financial interests to disclose.

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