

## He Knows Joy in All that He Does: on the Occasion of Jan Klein's 65<sup>th</sup> Birthday

Scientists working in Croatia constitute 0.17% of the world scientific community today but contribute only 0.08% of the world relevant scientific production (1). Two decades ago Matko Marušić, at the time the Associate Professor at the Department of Physiology, Zagreb University School of Medicine, offered the strategy – a so-called “scientific leap” – to speed the development of science in Croatia (2). He followed J. H. Comroe's saying that “the first step in recruiting a talented, creative scientist should be getting him involved in science” (3). Jan Klein from Max-Planck Institute for Biology in Tübingen was the first world scientist Prof. Marušić contacted in his effort to educate young Croatian scientists (2). I was one of the first students sent to his laboratory and what follows is my personal account of the experience of work with Jan Klein.

*Difficulty is an obstacle to the weak, a stepping stone to the strong*

*(Abraham Lincoln)*

Inspired by his grandfather's herbarium, Jan Klein developed a keen interest in botany. He had known that he was going to be a scientist since he was 8 years old (4), although there had been no external stimulus to his scientific interest in nature. His ancestors were farmers, and he was to be one, too. A young teacher of Klein, an enthusiastic nature lover, convinced Klein's mother to send the boy to college, so Klein entered the Charles University in Prague and started preparing himself for the teaching career, hoping at the same time that he would be able to continue with his scientific interest. But teaching biology, chemistry, and sometimes physics, astronomy, and mathematics at a model school in Prague was so time-consuming that he could pursue neither botanical excursions nor any research activity. Still, he could read a lot and he discovered a new love – molecular genetics. After three years of teaching, he decided to apply for the position at the Czechoslovak Academy of Sciences to become a full-time researcher. In 1961, Klein embarked on a research career, but not in plant genetics as he had originally planned. He joined Milan Hašek (1925-1984), internationally known for his research on immunological tolerance and his charismatic personality, at the Institute of Experimental Biology and Genetics (5). Hašek was also known as the founder of the Prague School of Immunology. He was a pronounced communist, but he used his political power to provide shelter for his younger colleagues. The only thing Hašek seemed to be interested in was intelligence and enthusiasm.

Living in Orwellian Czechoslovakia brought Jan Klein in conflict with the state secret police. When Leonore and Leonard Herzenberg from the Department of Genetics at Stanford University Medical Center, who met Klein at the conferences in Brno and Prague in 1965, invited him to Stanford to work as a postdoctoral fellow, he accepted. In 1967 he was granted the permission to travel to the United States, probably because Hašek personally vouched for his return. After experiencing a few months of freedom in the States, Klein had to return to Czechoslovakia (4). The decision about going back home was difficult, but Klein felt he owed that much to Hašek. The situation in Prague was rapidly evolving into what was afterward called the “Prague Spring” of 1968. When the Soviets, practicing Brezhnev's “limited sovereignty theory” invaded and occupied Czechoslovakia on August 21, 1968, Klein happened to be abroad, at the International Congress of Genetics in Tokio. Herzenberg helped him to come to Stanford again instead of going back to Prague, saving thus Klein's scientific career and life (4).

*Felix qui potuit rerum cognoscere causas*

Jan Klein arrived to the United States with just one suitcase and started a new life there (4). He married Dagmar Bednarova, and they moved to Ann Arbor, Michigan, where Klein joined Donald C. Schreffler as a Research Associate at the Department of Human Genetics at the University of Michigan School of Medicine. During summer 1969, Jan Klein started trapping wild mice in the vicinity of Ann Arbor. Through 1970, he captured some 150 wild mice in an area of not more than 100 km<sup>2</sup> (6). These mice were mated in the laboratory with an inbred strain B10.BR (bearing H-2<sup>k</sup> major histocompatibility complex haplotype) to produce a series of congenic strains which later become known as B10.W (“w” stands for wild) (6). Each of these over thirty B10.W strains carries a different H-2 haplotype of a wild mouse but has received the rest of their genes from the inbred strain C57BL10/Sn. Klein established his own laboratory at the Dental Research Institute, across the central campus of the University of Michigan in 1970, after coming back from the Midwinter Conference on Immunology in Asilomar, California, where he had described the two-locus model of the H-2 complex.

In 1974, Klein moved to the Department of Microbiology at the University of Texas Southwestern Medical School at Dallas, Texas. The first issue of *Immunogenetics*, with J. Klein as the managing Editor

and George D. Snell (1903-1996), cofounder of the field of immunogenetics, as the editor-in-chief, appeared at the end of 1974. In 1975, Klein summarized the knowledge about the H-2 in a book entitled "Biology of the Mouse Histocompatibility-2 Complex. Principles of Immunogenetics Applied to a Single System". In the preface Klein described the immunogenetics as a huge iron bridge that needs constant protection against rust and regarded himself entitled to add a layer of the paint (7). While approaching the end of the bridge, he realized that the starting point had become rusty again. So he decided to go back and start over this endless job.

After five years in Dallas, Klein made a great move and came to Tübingen, Germany. He was appointed the Director of the Department of Immunogenetics and he chaired, also successfully, the Board of the Directors of all Max-Planck Institutes (MPI) in Tübingen until 1983. Back in Europe, Klein very soon proved the favorable influence of the beauty of the "splendid isolation of the Schwäbische Alb" on the scientific endeavors (8). He proudly announced that *Immunogenetics* has, by its impact factor, become No. 1 among the immunology journals, and No. 51 among all 2,800 scientific journals processed by the ISI by the end of 1977 (9). After Snell's retirement in 1980, Klein became the Editor-in-Chief of the *Immunogenetics*, keeping the position until 1997.

Department of Immunogenetics in Tübingen has, under Klein's leadership, developed into a superb research institution. Among 55 publications produced by 96 collaborators of MPI for Biology in 1982, 36 came from the Department of Immunogenetics (10). Papers coming from Klein's Department were appreciated highly and the editors were often "delighted to publish a paper... which represents yet another technical tour-de-force by the Tübingen group" (11,12). World leading immunologists, including Walter F. Bodmer, Avriel N. Michison, Cheila S. David, and Dietrich Götze, frequently visited the Department of Immunogenetics in Tübingen. In December 1980, on his way back from the Nobel Prize Ceremony in Sweden, G. D. Snell visited Tübingen and gave a lecture. A year later, Jean Dausset, a Nobel Laureate, also visited Klein's Department as a member of the Peer Review Committee invited for the evaluation of the work.

In 1982, J. Klein published another excellent book "Immunology – the Science of Self-Nonself Discrimination", which was delightful to read and an endless source of information (13). His immense knowledge of history, literature, and art Jan Klein applied to the benefit of the science because for him "art and literature and science are one", as they were for Aldous Huxley. His "love for nature fused with love for beauty" Klein has explained as "a cultural mutation, which appeared suddenly and spontaneously, linked from the onset to a strong esthetic feeling" (4). Among 396 photographs and drawings and 90 tables, Klein's book also included reproductions of the paintings by Ivan Generalić, Max Ernst, and Pieter Breughel (13). His book changed the view of immunology: "neither teaching nor learning of the immunology will ever be the same again" (14).

From his boyhood to his current position of the patriarch among somatic cell geneticists, population geneticists, immunologists, immunogeneticists, molecular geneticists, taxonomists, anthropologists, and fish biologists, Jan Klein followed the saying that "being the scientist is not an occupation but a vocation and science is a calling without an alternative, a summons one is compelled to obey, and no sacrifice is too great in its service. Evenings, weekends and holidays are the first things to be relinquished, but ultimately science becomes a person's whole life" (4). He has demonstrated that one has to master not only scientific methods but also has to know thoroughly and passionately the history and philosophy of his scientific field (15). But, he reminded gently "all those who confront immunology all too intensely that there are things to enjoy beyond the laboratory" (4).

*No pressure, no diamonds*

(Mary Case)

Klein's firmly held belief, which he has always followed, is that "it is better to have a wrong opinion than to have no opinion at all. A wrong opinion can help resolve a problem by stimulating a debate. No opinion, however, contributes nothing" (4). "In science, a dictate – any dictate – is unacceptable. Fashionable hypotheses are detrimental to scientific progress as they are accepted uncritically and dominate the field of study long after their usefulness has worn out" (16). This thought could have belonged to Klein. He himself has tailored many hypothesis and has been often the first to question them again and again, and replace them mercilessly with better ones because "in science, it is wrong to focus on one explanation and to ignore others". Describing himself as a boy, Klein says: "He fails most of the time, or he misidentifies some of the plants, but the frustration he experiences only serves to enhance his curiosity" (4). Sometimes his ideas appeared heretic and spectacular, but were always experimentally founded, elaborated, elegant, and convincing. To make the understanding of his hypotheses easier, Klein uses picturesque way of elaboration, usually with a refreshing historical perspective and humorous comments (17). Being an avid reader, Klein has been acquainted with probably every progress made in his and related fields of science. The authors whose statements too often could not be confirmed made him even to "to put several of them on a blacklist and not bother to read their papers" (18).

Klein is very fond of the laboratory atmosphere and he knows all the shortcomings and problems that can appear. For example, he is able to judge unmistakably the amount of time needed for an experiment. As an uncompromising advocate of the bench work, Klein can be straightforward and harsh on one trying to slow down the pace of the work in progress or to come to the results taking shortcuts. Klein has always demanded from his collaborators to demonstrate their ideas or suggestions tested in experiments (since "mouse has never lied"), rather than strengthening their arguments by their "convictions" or "feelings". Klein has valued his own time highly and considered the idle time the most expensive. Therefore, he has always insisted on planning

and could lose his temper with someone who was not being able to keep to the time-schedule.

*Nothing great was ever achieved without enthusiasm*

(Ralph W. Emerson)

Antonio Juretić from the Department of Physiology at the Zagreb University School of Medicine was the first Croatian to work at Klein's Department. He spent a year there and proved to be an excellent researcher (19-22). After that, Klein felt confident to invite a group of Marušić's former students from Zagreb, Split, and Rijeka (Fig. 1). There were 22 of them in total. This is a respectable number, taking into the consideration the fact that total population of Croatia is 4.7 million. For the Croatian scientific community, equally important was Jan Klein's visit to Croatia. Invited by Marušić, he and his collaborators gave stimulating lectures in Zagreb and exchanged experiences with Croatian immunologists (22-26).



**Figure 1.** Strategy of the scientific leap for Croatia: Damir Vidović, Matko Marušić, Jan Klein, and Ivica Vučak (from left) in Jan Klein's office at the Department of Immunogenetics in Tübingen, 1982.

Several of those 22 young Croatian physicians who worked in Klein's Department later developed into scientists with an international recognition and their success proved definitely profitable for Croatia. Although he knew that "not every trainee who seems a promising researcher will remain in the research", Marušić was disappointed with those of his former students who failed to "accomplish the task" and, upon their return, did not pursue further scientific career for whatever reasons (2). Nevertheless, they learned a lot in Klein's laboratory and that knowledge has been a gain for the Croatian scientific and medical community.

*The success is the sum of small efforts, repeated day in and day out*

(Robert Collier)

In October 1980, Matko Marušić offered me to join Klein's Department for Immunogenetics in Tübingen. I was his former student and, at that time, a general practitioner in Sukošan and Bibinje, the villages at the Adriatic Coast. The position was available after A. Juretić had returned to Zagreb. In an attempt to ease my anxiety about going there, Marušić told me not to worry because everybody knew little to

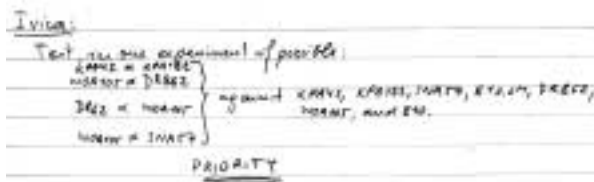
nothing about immunogenetics before joining Klein and his Department.

I got Max-Planck Society scholarship to carry out the research on "the role of I region in cell-mediated immunity". At first I had difficulties to establish the reproducibility of the results obtained in a series of the cell-mediated-lympholysis experiments. For quite some time, Klein was following my work with an expression revealing that he had no doubt about my complete lack of scientific technological experience. At that time, Hans-Georg Rammensee, another researcher in Klein's lab, was also investing a huge amount of time and effort in his laboratory work. He usually spent his evenings and nights in the laboratory, and his days with his wife and two kids at home, where he would find strength in the moments of impatience, failure, and sometimes even despair. Nevertheless, his results were soon recognized and his efforts paid off (27,28). Experiments that his group published in *Nature* in 1991, attracted a great attention and were cited 220 times to the end of 1992, making H.-G. Rammensee the most cited scientist in the 1991-1992 period (29,30).

The experiments done in the Department were regularly discussed and evaluated at the "work-in-progress" internal laboratory meetings. At first, these meetings depressed me. I could comprehend only a fraction of what they were talking about. The H-2, I-r genes, linkage disequilibrium, mixed-lymphocyte reaction, cell-mediated lympholysis, MHC (Major Histocompatibility Complex)-restriction, tryptic peptide mapping, and many other terms were totally new for me.

Every Friday afternoon, Klein would make a final tour of the Institute. After a short talk with each of us, he would collect the results of the experiments done during the week. Over the weekend, he would pass through the sheets with tables, analyze the data, and on Monday morning he would greet us with a twinkle in his eyes and new suggestions and questions to be tested during the week ahead (Fig. 2). We could often meet him in the Institute on Saturday mornings, when he devoted himself to reading, writing, and listening to classical music (preferably J. Haydn's 104 symphonies) in his office, without being interrupted by phone calls or other administrative nuisances. Those Saturday mornings resulted in a vast number of papers and several books in which he did not hesitate to express his personal opinion as he did in his witty commentaries in the "Odd Page" he introduced in the *Immunogenetics* (31).

The existence of the strain B10.STA62 carrying a new haplotype H-2<sup>w27</sup> with previously unidentified



**Figure 2.** Weekend is over, new suggestions are ready... A note from Prof. Jan Klein to Ivica Vučak.

$K^{w27}$  and  $D^{w27}$  alleles among the Klein's B10.W mouse colony played a decisive role in my research efforts (6,32). First results of my experiments I reported at the work-in-progress meeting approaching the Easter 1981. My data on the histogenetic CML analysis were concordant with serological and biochemical data already published, indicating that the STA62 strain carries a non-b allele at the  $E_{\beta}$  locus, ie, the presence of the class II genes  $A_{\alpha}^b$ ,  $A_{\beta}^b$ ,  $E_{\beta}^{w27}$  and  $E_{\alpha}^{w27}$  in the STA62 strain (6,33,34). The  $E_{\beta}^{w27}$  allele of the STA62 strain has been shown to be also functionally distinct from  $E_{\beta}^b$  allele (35,36). The class II genes  $A_{\alpha}^b$ ,  $A_{\beta}^b$ ,  $E_{\beta}^{w27}$  combination of the STA62 strain could have arisen either by mutation or recombination. Klein favored the latter process because a change of  $E_{\beta}^b \rightarrow E_{\beta}^{w27}$ , or vice versa, consisting of multiple nucleotide substitution and, hence, of multiple mutations, would require many millions of years. An intergenic crossing-over between the ( $A_{\alpha}A_{\beta}$ ) cluster and the  $E_{\beta}$  loci of two haplotypes, one of which was related to H-2<sup>b</sup>, Klein considered a more likely explanation. Being, thus far, the only haplotype derived by an intergenic crossing-over process the H-2<sup>w27</sup> confirms that the three loci in the  $A_{\alpha}A_{\beta}E_{\beta}$  cluster are very close perhaps even adjacent to each other. As the crossing-over does occur within the  $A_{\alpha}A_{\beta}E_{\beta}$  cluster therefore the designation of the segment as the "I-A subregion" and  $E_{\beta}$  locus as  $A_e$  is superfluous and without justification (34,37).

For me that was the best possible way to see what the science really was – a never-ending chain of efforts and attempts of many known and unknown contributors. I could not but agree with Snell that science is like a web, growing by interactions that reach out in time and space (38). One's own small place in this web is made possible by strands from the past and help of contemporaries.

Experience and publications I have earned in Tübingen and Prof. Matko Marušić's influence secured my position at the University in Zagreb (37, 39-42). But, blessing I have received by working in Klein's Institute lasted long after I have left it.

*There flows and flows, there flows a waterfall;  
My little drop in it, what does it mean at all?  
See, a rainbow in the water, it forms, it matters,  
It shines and glitters in thousands of patterns.  
This dream in the waterfall, that it could shine,  
Is also helped by the little drop of mine.*

*Dobriša Cesarić: The Waterfall  
(transl. by Miljenko Kovačiček)*

*Heu quanto minus est cum reliquo versari,  
quam tui meminisse!*

I was touched by the invitation to the "Origin of Antigen Presentation" symposium organized on the occasion of Jan Klein's birthday in Tübingen on July 19-20, 2001. As I was not able to come to that gathering of Jan Klein's friends, competitors, and opponents from the last 30 years I, sadly, missed the opportunity to meet again Hans-George Rammensee, Herbert Tichy, Werner Mayer, Felipe Figueroa, Dorothee

Wernet, Sofia Zaleska-Rutczynska, Zoltan A. Nagy, Zenro Ikezawa, Costas N. Baxevanis, Norihisa Ishii, Peter Walden, Sabine Adolph, Paul A. Singer, Sujay K. Singh, Piotr Kusnierczyk, Jacek M. Szymura and many, many others with whom I spent days in laboratory, which I remember with joy and pride.

It would not surprise me if Jan Klein became a Nobel Prize Laureate. He is a true scientist by the way he lives and by the way he enjoys in his work. Comparing his own feelings during a three-year-long writing of the book on immunology with those Charles Lindbergh must have had while trying to cross the Atlantic Ocean alone, Klein asked himself "should I endure, should the machine endure" (13). And he has endured. Knowing his vigor and elan, I can only join all those who wish Jan Klein, a "long-distance runner", many new races to enjoy in the fields of science (43).

Ivica Vučak

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