In the latter part of 1988, I was hired as a biochemist for a unique half-time research position in the Dept. of Dermatology of Amsterdam University by Drs Das and Westerhof. Ideal, because a revised Dutch ranking system brought positions that generally payed less than the babysitter cost. Initiating cultures on a Friday towards midnight, listening to news reports of the devastation on Tiannaman Square in Beijing I clearly found that there is no such thing as a half-time position in science. The new position in Amsterdam entailed figuring out how to culture skin explants and maintain tissue architecture. The organotypic culture protocol has certainly come in handy to answer many skin related questions. For example, we later proposed using explant cultures to study melanocyte migration (1). The next task was to ‘define a role for keratinocytes in vitiligo’. Vitiligo? What is that? Having knowingly encountered only a single vitiligo patient at the time, albeit a famous one in the field, I quickly realized many, if not most patients were hiding this surprisingly prevalent disease. Right from the start, it was amazing to find how very supportive the vitiligo patient group is of any research performed to understand the disease. Patients and controls came from everywhere, we prepared suction blisters while getting to know many patients personally. Painfully slowly, we built up a reservoir of melanocyte and keratinocyte cultures to be mixed to and fro. Clearly, melanocytes loved to be with keratinocytes, and the biggest problem was to get RID of melanocytes in keratinocyte cultures. Collaborating with Drs Nico Smit and Stan Pavel, I developed an interest in bleaching agents capable of selectively eliminating melanocytes. It didn’t take off at that point because instead we developed a simpler strategy to eliminate melanocytes in vitro based on limiting dilutions, but the collaboration did awake a long standing interest in bleaching phenols and the inherent dangers of melanization to melanocyte viability. During the agonizing wait for enough vitiligo melanocytes to grow I was persuaded to spend some time and effort looking into immunological properties of melanocytes. Based on a hunch: melanocytes look a little bit like Langerhans cells so perhaps they can do the same things, René van den Wijngaard and I started experimenting and a
few serendipitous findings opened up a whole new terrain. There was much to be discovered about melanocytes that turns out to be very relevant for vitiligo. Melanocytes secrete cytokines, as Dr Zalfa Abdel-Malek and the Krasagakes were already showing (2,3). Melanocytes were definitely capable of phagocytosis, as we first realized after observing melanocytes plated on a carpet of gold beads meant to show us all about melanocyte migration (4). Instead, the melanocytes stayed just where they were, but when left unattended for a week or two they ate away at the beads. Two micron beads otherwise used for FACS calibration served to quantify the phagocytic process by FACS analysis. Melanocytes revealed they were equally efficient as fibroblasts, known to routinely use phagocytosis for tissue remodeling (5). Even more exciting was the electron microscopic observation that melanosomes engaged themselves in melanosome-phagosome fusions, a function reserved for lysosomes in other cells (4)! That jived so well with reports of the melanosomes showing a striking resemblance to lysosomes in other respects—enzymatically, for example (6). Next we really wanted to know whether our findings were indicative of an antigen presenting function for melanocytes. Just imagine, with melanosomes taking on the task that lysosomes have in other cells, perhaps phagocytized antigens to be presented in the context of MHC class II molecules travel through melanosomes on their way back to the membrane. What does that tell us about the unique immunogenicity of melanocytic cells? And importantly, perhaps lysosomes packed with antigens can be transferred to neighbouring cells, just like melanosomes. You may never have seen it happen and neither have I, but that could be because lysosomes are not pigmented. The group of Dr. De Vries from Leiden helped us to demonstrate that melanocytes can process as well as present antigens (7). All the more interesting because vitiligo and melanoma offer the unique conditions where melanocytic cells actually express MHC class II molecules, and Dr. Ostrand –Rosenberg has in fact proposed to use this antigen presenting pathway to target melanoma cells (8). By now we were ready to take on sceptics and look for infiltrating immunocytes in vitiligo skin, to find whether the immunologic properties of melanocytes may be involved in the pathogenesis of vitiligo. Speaking with Dr Abdel Nasr at an ESPCR in Berlin it became clear that we had to be very precise about where and when to look for infiltrates in the skin. It made perfect sense: we now know that T cells are consistently found but only in actively depigmenting skin, just long enough to destroy a few melanocytes at the border and move on to more populated terrain (9). This was supported by our data that melanocytes are truly lost from vitiligo skin (10). Differentiated melanocytes are not found in the lesions, as we figured out with a panel of antibodies that will recognize melanocytes in different ways. At the time we were unaware of the relevance of the
molecules recognized by our antibodies- including NKI-Beteb reactive with immunodominant gp100-for immune recognition of melanocytic cells. We consistently found T cells and macrophages passing through depigmenting skin (9). The significance hit us when the groups of Rosenberg, as well as Boon closeby in Belgium, reported an unknown molecule named for its recognition by T cells (MART-1) was the first of a set of differentiation antigens efficiently recognized by melanoma-infiltrating T cells (11, 12). OK, so these cells recognize the tumor but they can respond to normal melanocytes as well! No wonder vitiligo is a positive prognostic factor for melanoma patients (13). In Amsterdam, Dr Anna Kalinska in the Das lab went on to demonstrate for the first time that T cells isolated from vitiligo skin indeed react with autologous melanocytes (14). Meanwhile, I joined Dr Raymond Boissy’s lab and moved to Cincinnati, convinced that we can understand more about why melanocytes come under attack in vitiligo patients but not in the general population, by studying gene expression in control versus vitiligo melanocytes. We did identify a gene differentially expressed in vitiligo melanocytes, and postulated that the VIT1 gene regulates expression of the hMSH6 mismatch repair gene (15). All the more exiting, because hMSH6 expression appears to be upregulated in response to radiation which appears to shift the T cell repertoire (16). Relevant for vitiligo? We’ll need to find out. Certainly, the genes Dr Spritz has since associated with vitiligo are very supportive of the autoimmune involvement in vitiligo (17). Together with Dr Luiten from Amsterdam and supported by data from the Giachino group in Italy, we are since learning that T cells in vitiligo skin will recognize the MART-1 antigen and gp100 (18). Analyzing T cell receptor genes together with Dr. Nishimura at the University of Chicago, we learned that that multiple clones invade vitiligo skin to support depigmentation (unpublished). And since T cells from vitiligo skin may be better at recognizing their targets than T cells in melanoma, the vitiligo TCR genes may well serve towards the treatment of patients with malignant melanoma. T cells can really do a number on melanocytes in vitiligo, and ‘stress’, that wonderful all-encompassing term, is proving to be an all-important precipitating factor for progressive depigmentation (19). Moreover, as Dr. Overwijk and others have convincingly demonstrated, anti-melanoma vaccines can induce depigmentation in mice (20). What a very big step forward it is to have an easily accessible mouse model of progressive depigmentation to model vitiligo in mice, supporting the data that Dr. Gisela Erf has been bringing to the table in the Smyth line chicken that spontaneously develops vitiligo (21). Will we be able to show that vitiligo can be precipitated in response to infection or to anti-infectious drugs, as Dr. Erf has shown in the chickens and as patients so frequently mention in their questionnaires? And can we develop the drugs needed to halt the
progression of vitiligo, using the depigmenting mice as a model? Can we make use of what we know to work in melanoma vaccines in order to help vitiligo patients, just as we are moving to isolate T cell receptor genes from vitiligo patients in order to target melanoma tumors? Many questions remain to be answered about vitiligo, and it has been enormously gratifying to work in such an exiting field with so much yet to be discovered, and to collaborate with so many sharp minds and hard working people to unravel the mysteries of vitiligo. And melanoma. The difficulties in actually bringing a cure lie mainly in limitations to funding, in part due to wars that cut further into the already limited resources of science. I have concerns about the future of science that I’m sure many of you share as well. All the more important to strengthen our collaborative efforts and share our joint ideas at meetings of the PanAmerican meetings for Pigment Cell Research. After an exciting meeting scheduled for Cincinnati this year, Vijay Setaluri and I plus other members of the local organizing committee hope to welcome all of you to Chicago for lectures scheduled by Dr Alan Houghton, Dr. Barbara Gilchrest, Dr. Heinz Arnheiter, Dr. Nina Jablonski, Dr Mitch Denning, Dr. Mary Hendrix and hopefully by…you in September of 2007. We hope you will share your thoughts on ‘Pigmentation and Diversity’ with us.

References: