



## In memoriam

Valerie J. Smith

February 17, 1953 – May 2, 2019



Valerie J. Smith passed away on May 2, 2019 at the age of 66 years. She held the position of Reader at The University of St Andrews in Scotland. I met Valerie (Val) for the first time in the spring of 1981, when she attended a course at Uppsala University. At that time, she was a lecturer at the Marine Biological Station at Millport on the Isle of Cumbrae, in the Firth of Clyde, Scotland. This station was then shared by the Universities of Glasgow and London. Val had recently completed a PhD on the shore crab and its immune defence with Professor Norman Ratcliffe at University College Swansea. I also worked with crustacean immunity using crayfish so we decided to meet once again to discuss possible future collaborations. I therefore visited Val in Millport in November 1981. We started an intense and long-lasting collaboration where I worked in Millport several times and Val came to Sweden several times. At the Marine Biological Station, we worked long hours but we always stopped just in time before 10 pm so we could get dinner and a beer at the closest pub. In those days the pubs closed at 10 pm and you could sit in the pub to 10.20-10.30.

At that time, it was very difficult to perform studies on immunity since you could basically only do *in vivo* studies, so we set out to isolate the different hemocyte types and try to make primary cultures of them, thus allowing studies *in vitro* and of cell to cell interactions during an infection. We worked with several different media and buffers to avoid aggregation and degranulation of the hemocytes. After extensive tests, we found that a low pH at ca 4.6 and a buffer with EDTA as an anticoagulant followed by gradient centrifugation using a new separation medium called Percoll (named after the discoverer of this medium, Håkan Pertoft) permitted the isolation of the different hemocyte types. We then found that the number of different hemocyte types varied a lot between diverse decapod crustacean species, something which we still do not fully understand the reason for. This method we published in 1983. This isolation method allowed us to study hemocyte behaviour after exposure to, for example beta-1,3 glucans, as well as the process of

the release of vesicles containing the proPO-system. This method was then applied with some slight changes for most invertebrates and the method is still in use by us and several other research groups.

In the 1980s-1990s, it was very popular to work with antibacterial peptides (AMP) and Val discovered a 11.5 kDa antibacterial peptide which she characterised from the hemocytes of the shore crab, *Carcinus maenas*. The results were published in 1999 in the European Journal of Biochemistry (now renamed FEBS Journal). This antibacterial peptide was several years later named "carcinin" by Val. This AMP was cysteine-rich, hydrophobic and showed specific activity against Gram-positive bacteria. This group of AMPs was later named crustins, members of which have an amino terminal glycine rich repeat region of 40–50 amino acid residues, in addition to the cysteine-rich C-terminus. More than 50 crustin sequences have been reported from a variety of decapods, including crab, lobster, shrimp and crayfish but also in ants. The crustins are mostly constitutively expressed in hemocytes, but their expression can be enhanced 2–3 times upon bacterial challenge in shrimp and lobster. It is interesting that crustin-like putative antibacterial proteins have been found in American lobster, Chinese shrimp, Brazilian penaeid shrimp, and black tiger shrimp. These proteins may also perform additional functions, perhaps as immune regulators in recovery from wounding, trauma or physiological stress. So, Val was the first who isolated and characterized crustins. In a recent paper (2017), Val and Liz Dyrinda found higher levels of carcinin or crustin in ovaries as well as some oocyte and eyestalk peduncles. If the eyestalks were ablated, the degree of carcinin increased in the healing peduncle, which may suggest that the protein can be associated with wounding, cell damage and/or tissue regeneration.

In recent years, Val studied a process called ETosis, which means a release of chromatin from the nuclei of inflammatory cells that entraps and kills microorganisms in the extracellular environment. This process is important for innate immunity in vertebrates and in an interesting paper in Nature Communications (2014), Val and colleagues showed that ETosis occurs in several invertebrate species, and that it probably predates the evolution of the coelom. In invertebrates, the released chromatin participates in defence and haemocytes could adhere and assemble on this "matrix" during encapsulation of foreign particles. They concluded that ETosis can be a very ancient process.

For a couple of decades, the possibility that some sort of specific or adaptive immunity is present in invertebrates has been discussed and debated among scientists. Val was very brave to write a review together with Chris Hauton in Bioessays in 2007 where they analyzed evidence for and against the presence of this process. They titled the review "Adaptive immunity in invertebrates: a straw house without a

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mechanistic foundation” and this maybe summarizes their opinion about this at that time.

Val served as President for the Society International Society of Developmental and Comparative Immunology, and she actually took over being President after me in 2009. She served for many years on the Editorial Boards of Developmental and Comparative Immunology and Fish and Shellfish Immunology. She was a very positive reviewer who always wrote detailed and constructive reviews, and she always promoted and supported young researchers.

Val had an extremely good sense of humour and in the early days we had lots of fun when we worked together. She will be missed by our community of comparative immunologists and by myself and my family, whom she met several times. I will personally miss Val a lot, but I can say to her, well-done Val.

**Kenneth Söderhäll**

I first met Val when she attended my lectures for her joint degree in Zoology and Microbiology at Swansea University during the 1970s. In those days, over 40 years ago, life was simpler with no research and teaching assessments or worries about funding so more time was available to enjoy life and inspire students.

Val was certainly inspired by her studies and she decided to continue further by taking a PhD with me. I was only too happy to advise her as she was obviously a lively and intelligent individual with the determination to succeed despite the very limited resources in the Zoology Department at that time. I also had been awarded a PhD studentship for studies on crab cellular immunity by the Natural Environmental Research Council (NERC), UK for which Val was the ideal candidate.

Why the interest in Crab immunity at that time? I was already researching insect immunity and attended a Society of Experimental Biology meeting in Swansea in 1974/5 and was actually excited to meet Dr. Spencer Davies the author of “Cytology of *Carcinus* haemocytetes and their function in carbohydrate metabolism. *Comparative Biochemistry and Physiology*, 46A, 569–581”. This meeting fired interest in Crustacean immunity which was rapidly conveyed to Val.

Val joined the Comparative Immunity group in Swansea which included Andrew Rowley, Steve Gagen and Adrian Schmit in the mid 1970s. She fitted extremely well into this diverse group with her lively sense of humour and tolerated the eccentric behaviour of the “boys”, for example, repairing a bent car fender by tying a rope to a lamppost and going into reverse! There was much laughter with these opposing personalities.

One outstanding memory was on Good Friday in 1976/77. We had recently been awarded a Zeiss Photomicroscope by The Royal Society and the Zoology Department had been given the funding to buy an Akashi Electron Microscope. Val used these to good effect to describe the *Carcinus* blood cells and immune reactions. We were granted permission to use the EM over Easter and decided to look at the nephrocytes of the crab at high magnification as they contained peculiar inclusions. The Akashi was very primitive as there was no condenser lens so that the higher the magnification the duller the image. Anyway, we started looking at a nephrocyte through the dull fog image and suddenly saw an unbelievable sight. In the centre of the cell was a crystal forming a perfect cross! We were both delighted and took it as a sign of things to come which turned out to be true for Val's career. This EM graph I believe was never published but certainly is in her PhD thesis. It would have been good to have entered this for a competition for photos of Biology in Art or Art in Biology.

Needless to say, Val finished her PhD thesis on time and sailed through her viva with Professor Ernest Naylor acting as her external examiner. I remember her NERC sponsors being very pleased with her work and publications at the time. Subsequently, she published a number of innovative papers in the late 1970s and early 1980s based on her PhD work. What happened next was an appointment to London

University and a subsequent outstanding collaboration with Professor Kenneth Söderhäll, University of Uppsala.

I guess the rest of her career is covered by Kenneth and maybe by Liz Dyrinda, her very dear friend and collaborator.

I stayed in touch with Val off and on over these many years. I always remember her commenting to me that one day she was telling PhD students how they should work hard and approach their research and she suddenly realised that she was telling them exactly what I had told her so many years before!

My last contacts with Val were when I congratulated her on her Nature paper and, subsequently, when she visited me in Rio de Janeiro with Liz Dyrinda about 2 years ago. We had a mini-symposium in which they presented their work. She was the same Val, lively, strong opinionated and laughing a lot. I had no idea she must have been so ill but this was never mentioned. I will sadly remember her from this meeting as she basically had found time with Liz to visit me despite her illness.

Needless to say, she will be missed by her many friends and by the international scientific community and especially by the comparative immunity group to which she contributed so much.

**Norman Ratcliffe**

As an undergraduate at Swansea, and a postgraduate in Norman Ratcliffe's group, I'd naturally heard of Val long before I met her. Val was also a Swansea alumna and had an enduring reputation in the Zoology Department for being fun, feisty and never suffering fools. She was also renowned for going immediately into a University of London lectureship after her PhD, instead of spending time as a post-doc. The downside of the London appointment was being posted to the University's marine biological station at Millport. While picturesque, Millport was a small town on a Scottish island of less than 12 km<sup>2</sup>, populated by less than 1,400 souls, not a natural choice of location for someone in their twenties. Val seized every chance to further her career by leaving the island at each conceivable opportunity to go and work elsewhere. It was good fortune that one of those openings led to Val and Kenneth Söderhäll working together, with their collaboration making major break-throughs in crustacean immunology. After a decade, Val moved to the University of St Andrews, where she remained for 30 years until her untimely death.

When I met Val at my first ISDCI conference in Wageningen, I found that while she lived up to her Swansea reputation, she was also extremely kind and totally committed to her PhD students, some of whom were there giving their first presentations. Unlike some established scientists, Val would talk to everyone regardless of their ‘importance’, and would always encourage students, however nervous they might be by attending a conference. One of Val's greatest strengths as a scientist was her ability to nurture and bring out the best in her students, many of whom have gone on to have their own successful careers in science.

Later, I was lucky enough to be part of Val's team bidding at the Cairns ISDCI for the next Congress, to be held in 2003. When we won the bid, we had some wild celebrations in the bar, but on our return, started booking rooms, approaching plenaries, and most importantly, planning the social events. Val had a clear vision of what she wanted, which included a fun run, an aquarium reception, pipers, a falconry display, and a banquet at Stirling Castle. Val admitted that the only time she felt nervous was writing the cheque to hire the Castle, as the deposit alone cost more than her first house. The banquet was a highlight and unique in ISDCI history to have almost all the men sporting kilts, leading Val to quip during the speeches “finally, we got the men in skirts”. It's a tribute to Val that St Andrews 2003 was still talked about years later as one of the best ISDCI meetings ever.

Val was an inspirational scientist, who had no time for sloppy or careless thinking, and who always looked ahead to the bigger picture. During the time I had the privilege of working with her, the best example of this was the Robb et al publication in Nature Communications.

Val, myself and Adriano Rossi were working on cell death in crustaceans with our PhD student, Calum Robb. Over lunch one day, Adriano told us about mammalian neutrophils and their extracellular chromatin traps which snare pathogens. For Val, that was a lightbulb moment, when she realized that this was something that invertebrate phagocytes must be capable of doing and made sense of all she'd observed with crustacean haemocytes. From then on, we worked to demonstrate this using Val's favourite lab animal, the crab, *Carcinus maenas*. While we could have published just the crab results, Val insisted on going further by repeating the experiments on a bivalve, but most importantly, on cells from an acoelomate. Besides the excitement of the lab work, as ever with Val there was humour throughout: this ranged from the time three academics with eight degrees between them couldn't manage to remove the lid from the cytocentrifuge head; to the evening we all got locked after hours in the aquarium and no-one could remember the security code to get out; to the competition to see how many times the word 'remarkably' could be squeezed into the manuscript.

Val enjoyed a remarkable career, but what made it exceptional was the context of the times that she worked in. When Val was first appointed, it was legal to ask women at interview about their intentions to get married and have children. At Millport, she was blocked from going out on the boats to collect specimens because the boat crews refused to allow women on board. Gender discrimination was common throughout her working life, but as this became more covert, age discrimination emerged instead. Val overcame all such obstacles and as part of her trade union activities, was a vocal opponent of the 'employer justified retirement age' policy that the University wanted to implement. Val defied attempts to force her early retirement, ending her career as a PI on four research council projects. Typically, and poignantly, she had already started planning a retirement party before she became ill towards the end of 2018.

Val was truly an inspiring scientist, a generous collaborator and a caring and committed PhD supervisor. More than that, she was a loyal friend who would move mountains to help the people she cared about. She loved entertaining, gardening, opera and reading, as well as caring for her beloved cat, Lucy. I was honoured to be a close friend and collaborator of Val's and like many others, I will miss her greatly.

*Liz Dyrinda*

I don't know when I first met Val Smith; it seems that I've always known her. We bonded based on both being women studying invertebrate immunology at a time when most scientists, including comparative immunologists, were men. She always had a mischievous look about her, which always made me want to participate in her schemes and to cause or get into trouble. It may have been during the 1997 ISDCI Congress in Williamsburg Virginia USA when Sylvia Smith (shark complement), Val Smith, and I sat together during one of the sessions or at a dinner and let it be known that we were all sisters.

Sylvia was the oldest and Val was the middle sister. Given that we didn't look at all alike, it was really amusing that some believed our joke. However, the long-term outcome of this innocent prank was that Val and I always thought of ourselves as sisters. Given that I have no biological sisters, it was nice to have an academic sister with similar interests and research passions.

Based on my 35-year interest in UK history spanning dates prior to 1603, I consider the 2003 ISDCI Congress in Scotland that Val organized to be one of my top life experiences. The gala dinner has a required dress code of long gowns for women and either black tie or formal Scottish kilt attire for men. It was held at Stirling Castle that is perched atop Castle Hill, a crag with cliffs and views on three sides. Stirling Castle was the site of Scottish rule and residence of kings and queens from medieval times until James the VI of Scotland became James I of England and moved from Stirling Castle to London in 1603. The time frame was right within my historical interests, and the castle and the gala were just too wonderful to describe. I was in awe for the entire function. It was truly amazing that Val pulled off an historical feat of taking all of the Congress participants to visit and experience medieval Scotland in grand style.

In 2015, Val asked me to run for ISDCI President elect. It seemed that she had been planning this for some time, for a sister to follow a sister in the position. However, given my exhaustion near the end of our academic year, her timing was not optimal and I turned her down. Nevertheless, she had planted the idea and I subsequently agreed to run in 2018. As the current ISDCI President, I had been comforted to know that Val would be there to answer all and any question about ISDCI history and policy. She knew everything about the society and understood how the society should work. She started the policy of using ISDCI funds to support student and postdoc travel to the international meetings. She taught me how best to distribute funds when I served as Secretary of Education. More recently, she provided to the current executive committee essential documents that are key to ISDCI as an organization, and I don't know whether I could have obtained these documents without her. Her loss to our society, to the science of crustacean and invertebrate immunology, and to me as an academic sister is immense.

Within the last year, I received a notification that funding was available for researchers to visit and collaborate with faculty at St. Andrews. Based on my interest in netosis after observing it under uncontrolled conditions in sea urchin coelomocytes, I seriously considered a collaboration with Val to understand netosis regulation in marine invertebrate immune cells. It seemed to be a wonderful project to undertake after retiring from teaching to focus on research. It would enable another visit to Scotland and for Val to instruct me on how to undertake this project using echinoid coelomocytes. But I hadn't gotten around to emailing her about the idea....

*L. Courtney Smith*