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A (selective) history of Australian involvement in cytokine biology

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Abstract

This review focuses on contributions to cytokine biology made by Australians in Australia. It is clearly biased by my own experiences and selective recollections especially related to the colony-stimulating factors in which Australian involvement has been pre-eminent from discovery to clinical use. Nevertheless Australian scientists have also made profound contributions to other areas of cytokine and growth factor biology (including interferons, inflammatory cytokines, chemokines and epidermal, insulin-like and vascular endothelial growth factors) that are briefly described in this review as well as other chapters in this volume.

Of necessity this review of Australia's contributions to cytokine biology will reflect primarily those areas in which I have been personally involved with lesser coverage of areas outside my area of expertise. Even so I am certain to offend my colleagues with the selective memory and loss of memory that inevitably comes with advancing age. To all such colleagues I offer my apologies in advance and hope that in time they will write their own histories.

Because of space considerations I have chosen to only describe discoveries made by Australians in Australia. This does not do justice to the field because many Australians have gone on to make major discoveries in cytokine biology elsewhere in the world (Richard Stanley, Malcolm Moore, Marc Feldmann, George Morstyn, Maureen Howard, David Gearing, Fabienne and Charles Mackay amongst others come to mind) while important figures like Bryan Williams made major discoveries elsewhere before settling in Australia. I have also excluded, because of space limitations, Australian contributions to chemokine biology (despite major contributions by Charles and Fabienne MacKay, Shaun McColl, Ian Clark Lewis, Jean-Pierre Levesque and others), inhibins and activins (despite their discovery and major contributions from David deKretser, David Robinson and Jock Findlay at Monash University and Prince Henry's Institute in Melbourne), relaxin (despite its discovery and analysis by Hugh Niall, Peter Hudson, Geoff Tregear and Ross Bathgate at the Howard Florey Institute in Melbourne) and Mic-1 (discovered and analysed by Sam Breit at St Vincent's hospital in Sydney).

Certainly Australia's most important contribution to cytokine biology was the discovery of the colony-stimulating factors (CSFs). This included the creation of in vitro clonal assays to enumerate and classify hemopoietic progenitor cells; definition of their individual growth

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requirements; discovery, purification and cloning of the CSFs; identification of the cellular receptors; elucidation of their biological activities in vivo and participation in their clinical deployment. There are probably no other cytokine systems in which Australia's involvement has been so complete and so important from discovery to clinical utility.

1. Colony Stimulating Factors (CSFs)

The CSF story began in 1964 with Ray Bradley's observation (at Melbourne University's Physiology Department) that mouse bone marrow cells formed colonies when cultured in agar-medium in petri dishes but only if he included underlayers of certain tissues or tissue fragments. He crossed the road to the Walter and Eliza Hall Institute (WEHI) to discuss these observations with Don Metcalf and together they concluded that the colonies were likely to contain granulocytes and macrophages. (although they did not use this terminology until later on)¹. Contemporaneously Leo Sachs' group in Rehovot also discovered the colony formation assay although they used spleen cells and the constituent colony cells were misidentified as mast cells presumably because the macrophages had phagocytosed metachromatic agar granules^{2,3}.

The colony assay was important in defining hemopoietic lineages and enumerating the committed progenitor cells (colony-forming cells) but importantly it also provided a robust assay to identify and purify putative lineage-restricted growth factors –the Colony Stimulating Factors (CSFs). In support of the existence of a specific growth factor, colony-stimulating activity was identified in mouse and human sera as well as urine and elevated levels were detected in leukemic mouse and human sera and in infected individuals. Unlike other circulating regulators normally produced by a single organ (eg insulin by the pancreas, erythropoietin by the kidneys) it was surprising that extracts or medium conditioned by a wide variety of tissues all showed detectable levels of colony-stimulating activity. This raised concerns by some workers in the field that colony-stimulating activity may have been a virus or bacterium, a bacterial product such as endotoxin or simply an in vitro artefact. Indeed this fear was a constant concern for Metcalf and those working with him right until the purification and cloning of the CSFs.

1.1 Purification and cloning of the CSFs

The high levels of a CSF in human urine that stimulated the production of macrophage colonies from mouse bone marrow cells (first identified by Bill Robinson, a visiting scientist from Colorado, in 1967)⁴ meant that the first attempts at WEHI to purify a CSF were based on this source. A PhD student from Western Australia, Richard Stanley, undertook this task from 1969-1974 but this proved a daunting task because, as it turned out, the very high specific activities of CSF meant that even highly active fractions contained miniscule amounts of active protein that were very difficult to separate from contaminating proteins with the separative technologies then available. Stanley moved to Toronto in 1974 and subsequently purified the human urinary CSF as a 45kDa protein in 1975⁵. He also purified a mouse form of this CSF from L-Cell-conditioned medium which he termed CSF-1 (rather than the M-CSF used previously) in 1977⁶. Using a modified purification protocol involving as the last steps affinity chromatography with a monoclonal antibody and reverse phase high performance liquid chromatography (RP-HPLC) the purified human urinary CSF was sequenced with a gas phase sequenator and degenerate oligonucleotides used to clone the cDNA at Cetus Corporation in 1985⁷.

Another PhD student of Metcalf, John Sheridan demonstrated that colony-stimulating activity could be extracted from most tissues and organs and that, consistent with Metcalf's observation that endotoxin elevated CSF levels in mouse serum, extractable CSF was elevated in all organs from such mice. He showed that medium conditioned by lungs from

endotoxin-injected mice contained high levels of CSF that stimulated both granulocyte and macrophage colonies^{8,9} (unlike Stanley's CSF that stimulated only macrophage colonies). Sheridan began the purification of this GM-CSF and continued until 1973 when it was taken over, first by Jim Camakaris and then in 1975, by Tony Burgess. They published the purification of GM-CSF from this source using conventional chromatographic steps in 1977¹⁰. However the yields from this fractionation procedure were low and it was not until 1985 (with the help of Lyndsay Sparrow at CSIRO) that an improved purification procedure of the asialo form of GM-CSF using reverse phase high performance liquid chromatography (RP-HPLC) produced material of sufficient amount and purity to allow the N-terminal amino acid sequence to be published¹¹.

Of course this had been achieved somewhat earlier than 1985 and the N-terminal sequence was used by Ashley Dunn and Nick Gough (at the adjacent Ludwig Institute for Cancer Research - LICR) to design redundant coding oligonucleotides that could be used to hybridise with GM-CSF cDNA in a mouse lung library. They used this clone to enrich mRNA and showed that upon expression in *Xenopus* oocytes this mRNA produced biologically active GM-CSF¹².

Studies by Greg Johnson at WEHI had shown that certain cells produced CSF that could stimulate multiple blood cell lineages (including eosinophil, erythroid and megakaryocyte as well as the usual GM colonies) and termed this CSF Multi-CSF¹³. Despite concerted efforts, Robert Cutler in our group was unable to obtain pure protein until much later¹⁴ but at the same time other groups were attempting to purify a range of biological activities that in retrospect we now know to be the same as Multi-CSF or interleukin-3. Jim Ihle's group in Frederick, Md purified IL-3 in 1982 from WEHI-3B cell-conditioned medium and he and others (including Andrew Hapel and John Schrader) subsequently showed that it was identical to Multi-CSF, 20 α SDH-stimulating activity, P-cell-stimulating factor and mast cell growth factor^{15,16}.

Ian Young's group at the John Curtin School of Medical Research (JCSMR) at the Australian National University in Canberra also used WEHI-3B cells as a source of IL-3 mRNA but employed mRNA hybridisation to a cDNA library followed by translation in *Xenopus* oocytes to identify IL-3 cDNA clones. They used the 32D cell line that requires IL-3 for growth as the bioassay and published the cloning of IL-3 in 1984¹⁷.

In 1979 Tony Burgess, Don Metcalf and I published that human GM-CSFs could be cleanly separated as two different molecules (termed GM-CSF α and GM-CSF β) by hydrophobic interaction chromatography¹⁸. The biological properties described for these CSFs would be clearly identified today with GM-CSF and G-CSF, respectively. Tony Burgess then described in 1980 activity from the serum of mice injected with endotoxin that could terminally differentiate the WEHI-3B leukemic cell line and that co-migrated with a CSF selectively stimulating granulocytic colonies¹⁹. I then undertook with Don Metcalf and other colleagues to purify the WEHI-3B-differentiating activity from the same endotoxin-stimulated lung source that had been used to purify GM-CSF.

As for other CSFs this turned out to be a long and somewhat tortuous exercise with continuous monitoring of all fractions for both CSF and differentiating activities. A major stumbling block was the complete loss of all activity as fractions became purer and protein concentrations became very low. This turned out to be due to non-specific adsorption of protein to plastic surfaces and could ultimately be completely prevented by the addition of low amounts (0.02%) of the detergent Tween 20. The final purification reported in 1983 resulted in over a million-fold purification of the activity but only a few micrograms of

purified protein from large batch sizes. The purification did however prove the identity of the differentiation-inducing activity with G-CSF²⁰.

Although we were able to show the existence of a human G-CSF with equivalent properties to that in the mouse and with complete cross-species reactivity²¹, human G-CSF was first purified, sequenced and cloned independently by Shige Nagata in Japan²² and Larry Souza and Malcolm Moore in the United States²³.

What is now known as interleukin-5 was first described as a T-cell replacement factor that stimulated B-cells in mice but was independently described as an eosinophil colony-stimulating factor by Don Metcalf in 1983²⁴ and an eosinophil differentiation factor by Colin Sanderson in 1985, then at the National Institute for Medical research in London. Takatsu and Honjo in Japan cloned IL-5 as a B-cell growth factor in 1986²⁵ while Sanderson collaborated with Ian Young (at the John Curtin School of Medical Research at the Australian National University in Canberra) and used direct expression in *Xenopus* oocytes to clone mouse and human IL-5 as an eosinophil growth factor in 1987 and 1988^{26,27}. The latter also demonstrated that human IL-5 unlike its mouse counterpart had no B-cell stimulating activity although both retained eosinophil-stimulating activity.

All the above colony-stimulating factors had been purified and cloned based on their ability to stimulate progenitor cells to proliferate and differentiate into mature hemopoietic cells. The first study to show a separate activity of CSFs in stimulating the functional activities in mature blood cells was by Emanuela Handman and Tony Burgess at WEHI in which they showed macrophages stimulated with GM-CSF had enhanced capacity to kill intracellular *Leishmania* parasites²⁸. John Hamilton (then in New York) and Burgess then showed that both GM- and M-CSFs stimulated plasminogen activator activity of macrophages²⁹. Mathew Vadas and Angel Lopez (at WEHI and then at the Institute of Medical and Veterinary Science (IMVS) in Adelaide extended these studies to show that several different CSFs (GM-CSF, G-CSF, IL-3, IL-5) could activate a number of different functional activities of human neutrophils, macrophages, eosinophils and basophils including antibody-dependent cell killing, phagocytosis and superoxide production³⁰⁻³⁵. They also showed with Glenn Begley at WEHI that very low doses of the same CSFs enhanced the survival of these mature cells³⁰. These initially unexpected findings have important consequences for the use of CSF antagonists in inflammatory and autoimmune diseases.

Soon after the cloning of the CSFs Ashley Dunn, Ed Stanley and Graham Lieschke at the Ludwig Institute in Melbourne embarked on a program to delete the genes in mice to determine their unique *in vivo* roles³⁶⁻³⁸. They showed an important but not unique role for G-CSF in maintaining steady state neutrophil numbers, hemopoietic progenitors in the bone marrow and the ability to respond to some bacterial infections. They also showed that GM-CSF deficiency resulted in lung pathology similar to pulmonary alveolar proteinosis and susceptibility to infection. These studies however also revealed redundant roles of GM-CSF and G-CSF in the generation of most hemopoietic cell types.

On the other hand Don Metcalf, Glenn Begley at WEHI and Richard Lang at LICR showed that the recombinant CSFs were active in stimulating granulocyte and macrophage production and inflammatory activation when injected into mice or expressed transgenically³⁹⁻⁴¹. Additionally John Hamilton and Iain Campbell at the University of Melbourne and Ian Wicks at WEHI used GM-CSF and G-CSF knockout mice or antibodies to show that both cytokines were essential for generation of disease and pain in collagen-induced arthritis and in osteoarthritis models in mice⁴²⁻⁴⁵. Antibodies to these cytokines or their receptors are currently being developed for the treatment of rheumatoid arthritis.

1.2 Clinical studies of the CSFs

One of the first clinical trials of G-CSF was a Phase I/II study in cancer patients receiving the myelosuppressive chemotherapeutic drug, melphalan⁴⁶. As a result of the local expertise in CSF biology, Amgen decided to conduct this trial at the Royal Melbourne Hospital under the leadership of George Morstyn (then at the Ludwig Institute and Royal Melbourne Hospital, RMH) and the trial demonstrated the ability of injected G-CSF to elevate neutrophil levels in the blood and to decrease the period of neutropenia following chemotherapy. This decision by Amgen proved to be wise because during this trial patient samples were analysed by Don Metcalf and Uli Duhrsen at the Hall Institute and they found that, unexpectedly, G-CSF also caused a dramatic mobilization of progenitor cells out of the bone marrow into the blood⁴⁷. This was important because Chris Juttner and Bik To working in Adelaide had previously shown that progenitor cells mobilized into the peripheral blood after chemotherapy could be used to achieve autologous hemopoietic reconstitution that was more rapid than that seen with bone marrow transplants⁴⁸. This was confirmed for G-CSF-mobilised stem cell transplants⁴⁹ and these observations were the basis for the current use of G-CSF-mobilized peripheral blood stem cell transplants to replace traditional bone marrow transplantation.

Similarly, GM-CSF was first trialled in Melbourne by Graham Lieschke Richard Fox and George Morstyn (at LICR and the RMH) in cancer patients receiving myelosuppressive chemotherapy where it was shown that GM-CSF elevated all white blood cells in the blood but had somewhat more serious toxicities compared to G-CSF⁵⁰. Again in parallel with the effects of G-CSF it was subsequently shown by Jean-Luc Villeval and Duhrsen at WEHI that GM-CSF could also mobilize progenitor cells into the blood⁵¹. George Morstyn was ultimately recruited to Amgen where he headed clinical development.

1.3 CSF receptors

With the tiny amounts of purified G-CSF available in 1983 I explored iodination procedures to radio-iodinate it and identify its cellular receptors. Conventional procedures caused oxidative damage and inactivation of the protein but a two-phase iodination procedure allowed preparation of high specific activity radiolabeled G-CSF that allowed us to identify receptors on normal myeloid cells and cell lines. There were surprisingly small numbers (a few hundred) of a single class of high affinity receptors detected on myeloid cells and myeloid cell lines but not on lymphoid, erythroid or other cells^{52,53}. I then used similar techniques to identify Multi-CSF (IL-3) receptors on a broader range of hemopoietic cells⁵⁴.

Tony Burgess at LICR first described the existence of both high and low affinity specific receptors for GM-CSF on mouse hemopoietic cells with small numbers of high affinity receptors per cell⁵⁵. This study and a subsequent study with Francesca Walker also showed that other CSFs could partially compete for binding of GM-CSF but only to high affinity receptors⁵⁶ and this foreshadowed the discovery of a common shared receptor chain required for conversion of low-affinity CSF receptors to their high affinity forms.

I was struck by the power of microscopic autoradiography to delineate receptor-positive from negative cells in the above studies and decided to apply this technique to the screening of a human placental expression library to detect human GM-CSF receptors. David Gearing and I used cDNA pools from this library to transfect COS cells and ultimately identified a cDNA clone that conferred low affinity GM-CSF binding⁵⁷. We identified sequence features that identified a new class of cytokine receptors (along with those for erythropoietin, IL-2 and prolactin) and postulated that a separate adaptor subunit might explain both the generation of high affinity receptors and ones that cross-reacted with high affinity receptors for IL-3. Indeed, Doug Hilton at WEHI went on to use the conserved

sequence elements (particularly the WSXWS sequence) to probe for new cytokine receptors and succeeded in cloning the specific receptors for IL-11 and IL-13^{58,59}. The adaptor subunit (common beta chain) was cloned by Atsushi Miyajima in Japan⁶⁰ and shown to be an affinity converting subunit for GM-CSF, IL-3 and IL-5 receptors.

Angel Lopez in Adelaide, whose binding studies in various human cells had also foreshadowed the existence of a common beta chain receptor for these cytokines went on to define the common structural features of GM-CSF, IL-3 and IL-5 that allowed binding to an identical site on the common beta chain⁶¹ and to generate antibodies targeting this site on the common beta chain⁶². In 2008 he and Michael Parker (at St Vincent's Institute in Melbourne) determined the x-ray crystallographic structure of the tripartite GM-CSF receptor complex revealing an unexpected arrangement of the subunits in a hexameric structure but indications that a dodecamer had to form to bring putative associated JAKs into apposition so that signalling could occur⁶³.

2. Other Cytokine Receptors

Mike Waters at the University of Queensland first described growth hormone binding to receptors in rabbit liver and to a serum binding protein. In collaboration with scientists at Genentech he showed that both purified proteins contained the same amino acid sequence and this allowed cloning of both rabbit and human growth hormone receptors⁶⁴. Waters later developed a model for growth hormone-dependent signaling through the receptor that involved pre-formed homodimers of the receptor (rather than ligand-induced homodimerisation) that are induced to rotate and move vertically relative to each other by ligand binding so that pre-associated JAKs are then able to cross-phosphorylate each other and initiate intracellular signaling⁶⁵.

Richard Simpson at LICR purified the extracellular domain of the interleukin-6 receptor, demonstrated that the stoichiometry of the receptor complex was 2:2:2 (IL-6: IL-6R α :gp130) and with Jose Varghese at CSIRO determined the x-ray crystallographic structure of the extracellular domain of IL-6R α ⁶⁶.

Tom Garrett and Jian-Guo Zhang at WEHI determined the structure of LIF in complex with the LIF receptor extracellular domain⁶⁷ and with Yibin Xu the structure of the full-length extracellular domain of gp130⁶⁸. The former structure showed a non-canonical interaction of the cytokine with a central immunoglobulin domain rather than the classical 'cytokine-binding domain' of cytokine receptors and the latter showed for the first time how the three fibronectin III domains of the tall cytokine receptor legs came together adjacent to the transmembrane region of the receptor.

3. IL-6 family and other inflammatory cytokines

During the purification of G-CSF as the factor inducing differentiation of the WEHI-3B myeloid leukemia cell line it became apparent to us that G-CSF had very different properties to a factor described by Leo Sachs' group in Israel that induced differentiation in a different myeloid leukemia cell line called M1. We imported the cell line and Doug Hilton and I initiated purification of the M1 factor from Krebs ascites medium. The purification of this Leukemia Inhibitory Factor (LIF) was performed in record time and the protein was sequenced by Richard Simpson at the Ludwig Institute. David Gearing and Nick Gough (then at WEHI) cloned mouse LIF cDNA using degenerate oligonucleotides corresponding to the amino acid sequence and subsequently also cloned the human cDNA⁶⁹.

Unlike G-CSF, LIF had no colony-stimulating activity so its physiological role was unknown at the time of cloning. Over the next few years multiple groups purified or cloned

their favourite biological activities only to find it was LIF. These activities included cholinergic neuronal differentiation, hepatocyte stimulatory activity and others. Lyndsay Williams at EMBL in Heidelberg had noted similarities between the factor he was trying to purify (that maintained embryonic stem cells in an undifferentiated state) and LIF and with Nick Gough showed that this was an intrinsic activity of recombinant LIF⁷⁰.

Richard Simpson collaborated with Jacques Van Snick (from the Brussels branch of the Ludwig Institute) to purify and clone a B-cell hybridoma and plasmacytoma-stimulating factor that proved to be identical with interleukin-6^{71,72}.

Jenny Gamble and Mathew Vadas (then at the Institute for Medical and Veterinary Science –IMVS- and Hanson Institute in Adelaide) revealed an important role of tumor necrosis factor (TNF) in inducing human neutrophil adhesion to endothelium (acting on expression of adhesion molecules on both neutrophils and endothelial cells) and its inhibition by TGF β ^{73,74}. They also identified the role of sphingosine kinase 1 in TNF signalling through its interaction with TRAF2⁷⁵.

Fabienne Mackay, while at Biogen in the U.S. had discovered the TNF-related cytokine B-cell Activating Factor (BAFF) and showed that mice transgenic for BAFF expression had excess levels of mature B and T cells as well as signs of on-going autoimmune reactions reminiscent of systemic lupus erythematosus. Subsequent work with Charles Mackay at the Garvan institute in Sydney showed that older transgenic mice developed a secondary autoimmune pathology similar to Sjogren's syndrome and that the mechanism probably involved survival of self-reactive memory B-cells in the periphery^{76,77}.

Jenny Stow at the Institute for Molecular biosciences in Queensland has explored the mechanisms of inflammatory cytokine secretion from macrophages and other cells. She showed that TNF is transported from the endoplasmic reticulum to the Golgi complex and then to recycling endosomes before fusion to the extracellular membrane and has identified many of the SNAREs (Syntaxins, VAMPs) as well as Rabs and PI3K isoforms involved at each step^{78,79}.

4. Cytokine Signaling

Andrew Wilks (then at the Ludwig Institute) used degenerate primers to highly conserved sequences in tyrosine kinases to amplify by PCR a novel kinase from a hemopoietic cell line⁸⁰. When he showed the sequence to Tony Burgess the unenthusiastic response was “so it's Just Another Kinase” and the acronym JAK stuck though it was latter attributed to being an acronym of Janus kinases to reflect the duplicated kinase domain. The full sequences of JAK1 and JAK2 were published in 1991⁸¹. Wilks, with Sandra Nicholson, showed that JAK1 bound to the G-CSF receptor and became tyrosine phosphorylated after ligand binding⁸² while collaborations with several international labs established the central role of JAK-STAT signalling for a variety of cytokines. After leaving the Ludwig Institute Wilks founded a company (Cytopia) to develop JAK inhibitors and in 2006 collaborated with Isabelle Lucet and Jamie Rossjohn at Monash University to produce the first JAK2 kinase domain structure with a bound inhibitor⁸³. This structure revealed a unique structural feature not present in other kinases, called the JAK insertion loop, that linked the G and I helices of the lower lobe of the kinase domain.

During the purification of LIF Doug Hilton had been struck by the potential of the IL-6/LIF-induced differentiation assay of M1 cells to be used as a positive selection screen for inhibitors of cytokine signalling. He and Robyn Starr used a retroviral expression library provided by Tom Gonda (then at the Hanson Centre/IMVS in Adelaide) to infect M1 cells and look for clones that failed to differentiate in the presence of excess IL-6. They found

such a clone and called the cDNA insert Suppressor of Cytokine Signaling 1 (SOCS1)⁸⁴. SOCS1 contained a central SH2 domain and a C-terminal domain (the SOCS box) conserved with CIS and subsequently found in six other SOCS proteins as well as several other classes of molecules with different protein-protein interaction domains⁸⁵. Jian-Guo Zhang at WEHI discovered that the SOCS box was a recognition element for binding elongins B and C thus recruiting E3 ubiquitin ligase activity to SOCS complexes leading to proteasomal degradation of bound proteins⁸⁶. Sandra Nicholson and Manuel Baca also at WEHI defined the specificity of the SOCS SH2 domains and showed the preferential targets, at least for some SOCS proteins, were tyrosine phosphate sequences in the receptor cytoplasmic domain⁸⁷ while a sequence just N-terminal to the SH2 domains of SOCS1 and SOCS3 could inhibit JAK activity directly⁸⁸.

Warren Alexander (with Doug Hilton, Don Metcalf, Ben Croker and Andrew Roberts) at WEHI led efforts to determine the in vivo roles of all the SOCS proteins by generating constitutive or conditional knockouts in mice. These studies unexpectedly revealed a great deal of specificity in the actions of SOCS proteins with SOCS1 selectively affecting interferon signalling, SOCS2 affecting growth hormone signalling and SOCS3 affecting signalling by G-CSF and IL-6-family cytokines⁸⁹⁻⁹². The molecular basis of this specificity was resolved in 2012 when Jeff Babon at WEHI showed that the SH2 domain of SOCS3 bound to a tyrosine phosphate sequence on the receptor while the reverse face of the SH2 domain and the kinase inhibitory region bound to a conserved sequence in the JAK insertion loops of JAK1,2 or Tyk2 and blocked substrate entry into the active site⁹³.

Matthias Ernst and Brendan Jenkins at the Ludwig Institute used various knock-in mutants of gp130 (the common signalling receptor subunit for IL-6, LIF, IL-11 and other cytokines) to dissect the relative contributions of strength of signalling by STAT3 versus MAP kinase to different biological phenomena. They showed that excessive STAT signalling with decreased MAPK signalling (gp130 Y757F mutant that prevents SHP2 activation and SOCS3 binding) resulted in inflammation and autoimmunity and IL-11-dependent gastric adenomas. On the other hand reduced or absent STAT-signaling with enhanced MAPK signalling (gp130 with STAT-binding sites mutated) resulted in impaired colonic mucosal wound healing and cartilage degeneration (synovial chondromatosis)^{94,95}.

Wally Langdon and Peter Klinken (both then at WEHI) identified the viral oncogene v-cbl in an acutely transforming murine retrovirus that causes early B-cell lymphomas⁹⁶ and, after moving to the University of Western Australia in Perth, Langdon went on to identify the normal cellular homologue (c-cbl) and, with various collaborators overseas, its roles in tyrosine kinase signalling. Like SOCS proteins c-cbl is an E3 ubiquitin ligase that contains protein recognition modules (including a phosphotyrosine-binding domain) and a RING domain that binds activated E2 enzyme. Its major substrates are receptor tyrosine kinases (eg EGF receptor) and T-cell receptor-associated tyrosine kinases (eg Zap-70) with cbl-mediated ubiquitination resulting in inhibition of activity⁹⁷.

David Vaux at the Hall Institute identified the role of the proto-oncogene Bcl-2 in maintaining hemopoietic cell survival following cytokine withdrawal⁹⁸. Subsequent studies by him and others at the Hall Institute led by Jerry Adams, Suzanne Cory, Andreas Strasser, David Huang and Peter Colman identified many aspects of the apoptotic pathway and its implications for cancer, immunology and autoimmune diseases⁹⁹.

Angel Lopez in Adelaide identified novel pathways involving serine phosphorylation of the beta chain common receptor for GM-CSF/IL-3/IL-5 that resulted in cell survival at very low doses of these cytokines. The pathway involved 14-3-3 protein binding and recruitment of

PI-3-kinase but the exact relationship of these pathways to anti-apoptotic signalling is still unclear¹⁰⁰.

David Bowtell while at the Howard Florey institute in Melbourne identified the mammalian homologue of *Drosophila* son of sevenless (*sos*) and showed that it linked SHC-Grb2, associated with activated EGF receptors, to activation of the Ras-MAPK pathway^{101,102}. Roger Daly while in New York with Josse Schlessinger had cloned Grb2 and demonstrated its role in Ras signaling. Upon his return to Australia at the Garvan Institute in Sydney Daly also cloned Grb14 and demonstrated its role in insulin receptor signalling and focussed on the roles of Grb2, Grb7 and the Grb2-interacting protein Gab2 in signalling by EGF and ErbB receptors, particularly in breast cancer¹⁰³⁻¹⁰⁵.

5. Interferons

Alick Isaacs began his work on viral interference at the Walter and Eliza Hall Institute where McFarlane Burnet had established the conditions for growing viruses on chicken chorio-allantoic membranes¹⁰⁶. He coined the term interferon for the interfering principle about seven years later¹⁰⁷.

Bryan Williams (in Toronto, Cleveland and then at Monash Institute of Medical Research in Melbourne) cloned and studied the regulation of protein kinase R, a Ser/Thr protein kinase induced by interferon and activated by viral double stranded RNA as well as inflammatory stimuli. PKR appears to be involved in multiple antiviral responses including effects on protein synthesis, NF- κ b activation, apoptosis and actin dynamics^{108,109}. Paul Hertzog and Ishmael Kola at the same Institute in Melbourne generated mice deficient in interferon α 1 receptors and showed these were required for type I antiviral responses as well as the inhibitory effects of interferons on macrophages¹¹⁰. Hertzog also went on to help define the role of SOCS1 acting on this receptor or its associated kinase Tyk2 in constraining interferon activity¹¹¹.

Hertzog and Williams with Ashley Mansell also made several important contributions to the involvement and regulation of innate immune Toll-like receptor signalling leading to interferon production¹¹²⁻¹¹⁴.

On the other hand Prue Hart and John Hamilton at the Dept of Medicine, Melbourne University discovered the important anti-inflammatory and polarising effect of interleukin-4 on human macrophages¹¹⁵.

6. Epidermal and Insulin-like growth factors

Rob Baxter at the Kolling Institute in Sydney has made numerous contributions to our understanding of the insulin-like growth factor binding proteins (IGFBPs). These include the purification and cloning of the acid-stable component of growth hormone-inducible IGFBP3^{116,117}, demonstration that IGFBPs could either enhance or inhibit IGF signalling depending on context, measurement of IGFBP levels by radioimmunoassay and demonstration of IGF-independent actions of IGFBPs¹¹⁸.

Colin Ward and Tom Garrett at CSIRO Melbourne solved the first X-ray structure of the first three domains -Leucine-rich domain1-Cysteine-rich domain-Leucine-rich domain2 (L1-CR-L2) of the IGF-1 receptor revealing domain structures expected to be conserved in insulin and other receptors¹¹⁹. Ward along with Neil McKern and Mike Lawrence (also at CSIRO) also solved the structure of the homologous insulin receptor ectodomain (L1-CR-L2-(FNIII)₃) dimer and showed a folded-over conformation in which insulin is likely to contact L1 of one monomer and the first FNIII domain of the other¹²⁰.

John Wallace and John Ballard (at the University of Adelaide and CSIRO in Adelaide) used mutants and structural analysis of IGFs to identify separate binding sites on IGFs for IGF-BPs on the one hand and type I receptors on the other^{121,122}.

Garrett, Ward along with Tony Burgess also solved the first structure of the extracellular domain of the epidermal growth factor (EGF) receptor in complex with transforming growth factor- α (TGF- α)¹²³. This structure contained the domains L1-CR1-L2-CR2 and showed that the monomers in each receptor dimer bound a TGF molecule each through contacts on L1 and L2 with dimerization occurring through long interdigitating loops of the corresponding CR1 domains and through CR2-CR2 contacts. Comparison of this structure with the unliganded monomer form showed dramatic movements of L1-CR1 upon ligand binding though how these movements and dimerization activate the cytoplasmic kinase domains remains elusive. These authors also showed that the related ErbB2 receptor takes on the conformation of the liganded EGF receptor (even though it binds no ligand) but forms only monomers and is thus poised to form active heterodimers when ligands bind to other ErbB-related receptors^{124,125}.

7. Vascular Endothelial Growth Factors (VEGFs)

Jonathan Stone at the University of Sydney with colleagues in Israel showed that VEGF was the critical survival factor for newly formed retinal blood vessels in vivo and that hyperoxia turned off VEGF expression in retinopathy of prematurity while hypoxia induced glial expression of VEGF during neural development^{126,127}.

Steve Stackman and Mark Aachen (then at the Ludwig Institute in Melbourne) identified VEGF-D as a new member of the vascular endothelial growth factor family based on homology searches. They showed it was mitogenic for endothelial cells and bound selectively to VEGF receptors 2 and 3¹²⁸. Subsequent studies by these authors showed that VEGF had both angiogenic and lymphangiogenic activities with the latter being an important component of lymphatic metastasis in some tumours¹²⁹ though not essential for normal lymphatics development in mice¹³⁰.

8. Concluding Remarks

It is fairly clear from the foregoing that Australians have made very significant discoveries and contributions to the field of cytokine and growth factor biology. Without being able to quantitate it, it nevertheless seems likely that this contribution is out of proportion to Australia's share of the research enterprise and it is interesting to consider why this may be so.

Certainly part of the reason for success was inspirational leadership as evidenced by Don Metcalf's central position in all of the major developments in the CSF story. This leadership included the ability to recruit very many highly talented individuals who brought disparate skills that were required for the biological, biochemical, molecular biological and clinical aspects required for the overall project and to convince each of them to pursue his overall vision. It also required incredible persistence through many difficult, challenging and dark periods filled with doubt – exemplified by Metcalf's subtitle for his historical account *Summon up the Blood – In dogged pursuit of the blood cell regulators*.

I believe another reason for success was a highly collaborative culture that marks Australian research. This was exemplified right at the beginning of the CSF story with Ray Bradley's willingness to share his initial results with Metcalf and to seek additional input. Collaborations within large groups working on a single problem are not easy because it is difficult to share equally the academic rewards but where it can be achieved through

inspired leadership relatively small groups can effectively compete with much larger groups. Throughout my career, where we have had wins, the wins were invariably only a few weeks or a few months ahead of our competitors - re-enforcing the efficiency of effective collaborations. These collaborations were also enhanced in Melbourne by the physical proximity of WEHI, LICR, Melbourne University, RMH and CSIRO in the 'Parkville Strip'. This provided ready and continuous access to an enormous array of skills, technologies and resources that were all used in the CSF and other stories.

Finally the diaspora of scientists who had worked with Metcalf at WEHI and then moved on to other States in Australia or elsewhere in the world provided several important clusters of activity where collaboration (and in some cases healthy competition) remained high.

The remaining chapters in this special issue of *Cytokine and Growth Factor Reviews* reveal that Australian contributions to cytokine biology will continue to be significant into the future.

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Biography

Professor Nicos Nicola is co-head of the Division of Cancer and Haematology at the Walter and Eliza Hall Institute of Medical Research in Melbourne. He obtained his PhD as a protein chemist under Sydney Leach in the Biochemistry Department at Melbourne University in 1976 and, after a postdoctoral stint with Gerry Fasman at Brandeis University in Boston, joined Don Metcalf and Tony Burgess at the Hall Institute where he remains today. He and his colleagues (including Doug Hilton) first purified the cytokines G-CSF and LIF, cloned receptors for GM-CSF, IL-11 and IL-13 and identified the SOCS family of cytokine signalling inhibitors. His current interests are in cytokine signaling and leukemia development.

References

1. Bradley TR, Metcalf D. The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med Sci. 1966; 44:287–99. [PubMed: 4164182]
2. Ichikawa Y, Pluznik DH, Sachs L. In vitro control of the development of macrophage and granulocyte colonies. Proc Natl Acad Sci U S A. 1966; 56:488–95. [PubMed: 5229970]
3. Pluznik DH, Sachs L. The cloning of normal “mast” cells in tissue culture. J Cell Physiol. 1965; 66:319–24. [PubMed: 5884359]

4. Robinson WA, Stanley ER, Metcalf D. Stimulation of bone marrow colony growth in vitro by human urine. *Blood*. 1969; 33:396–9. [PubMed: 5766776]
5. Stanley ER, Hansen G, Woodcock J, Metcalf D. Colony stimulating factor and the regulation of granulopoiesis and macrophage production. *Fed Proc*. 1975; 34:2272–8. [PubMed: 1081456]
6. Stanley ER, Heard PM. Factors regulating macrophage production and growth. Purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. *J Biol Chem*. 1977; 252:4305–12. [PubMed: 301140]
7. Kawasaki ES, et al. Molecular cloning of a complementary DNA encoding human macrophage-specific colony-stimulating factor (CSF-1). *Science*. 1985; 230:291–6. [PubMed: 2996129]
8. Sheridan JW, Metcalf D. Studies on the bone marrow colony stimulating factor (CSF): relation of tissue CSF to serum CSF. *J Cell Physiol*. 1972; 80:129–40. [PubMed: 5071877]
9. Sheridan JW, Stanley ER. Tissue sources of bone marrow colony stimulating factor. *J Cell Physiol*. 1971; 78:451–60. [PubMed: 5160835]
10. Burgess AW, Camakaris J, Metcalf D. Purification and properties of colony-stimulating factor from mouse lung-conditioned medium. *J Biol Chem*. 1977; 252:1998–2003. [PubMed: 300377]
11. Sparrow LG, Metcalf D, Hunkapiller MW, Hood LE, Burgess AW. Purification and partial amino acid sequence of asialo murine granulocyte-macrophage colony stimulating factor. *Proc Natl Acad Sci U S A*. 1985; 82:292–6. [PubMed: 3871523]
12. Gough NM, et al. Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte-macrophage colony stimulating factor. *Nature*. 1984; 309:763–7. [PubMed: 6610831]
13. Johnson GR. Colony formation in agar by adult bone marrow multipotential hemopoietic cells. *J Cell Physiol*. 1980; 103:371–83. [PubMed: 7400223]
14. Cutler RL, Metcalf D, Nicola NA, Johnson GR. Purification of a multipotential colony-stimulating factor from pokeweed mitogen-stimulated mouse spleen cell conditioned medium. *J Biol Chem*. 1985; 260:6579–87. [PubMed: 4039729]
15. Ihle JN, Keller J, Henderson L, Klein F, Palaszynski E. Procedures for the purification of interleukin 3 to homogeneity. *J Immunol*. 1982; 129:2431–6. [PubMed: 6815268]
16. Ihle JN, et al. Biologic properties of homogeneous interleukin 3. I. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, p cell-stimulating factor activity, colony-stimulating factor activity, and histamine-producing cell-stimulating factor activity. *J Immunol*. 1983; 131:282–7. [PubMed: 6190911]
17. Fung MC, et al. Molecular cloning of cDNA for murine interleukin-3. *Nature*. 1984; 307:233–7. [PubMed: 6420702]
18. Nicola NA, Metcalf D, Johnson GR, Burgess AW. Separation of functionally distinct human granulocyte-macrophage colony-stimulating factors. *Blood*. 1979; 54:614–27. [PubMed: 313822]
19. Burgess AW, Metcalf D. Characterization of a serum factor stimulating the differentiation of myelomonocytic leukemic cells. *Int J Cancer*. 1980; 26:647–54. [PubMed: 6972358]
20. Nicola NA, Metcalf D, Matsumoto M, Johnson GR. Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. *J Biol Chem*. 1983; 258:9017–23. [PubMed: 6190815]
21. Nicola NA, Begley CG, Metcalf D. Identification of the human analogue of a regulator that induces differentiation in murine leukaemic cells. *Nature*. 1985; 314:625–8. [PubMed: 2986009]
22. Nagata S, et al. Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature*. 1986; 319:415–8. [PubMed: 3484805]
23. Souza LM, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science*. 1986; 232:61–5. [PubMed: 2420009]
24. Metcalf D, Cutler RL, Nicola NA. Selective stimulation by mouse spleen cell conditioned medium of human eosinophil colony formation. *Blood*. 1983; 61:999–1005. [PubMed: 6187399]
25. Kinashi T, et al. Cloning of complementary DNA encoding T-cell replacing factor and identity with B-cell growth factor II. *Nature*. 1986; 324:70–3. [PubMed: 3024009]
26. Campbell HD, et al. Isolation, structure and expression of cDNA and genomic clones for murine eosinophil differentiation factor. Comparison with other eosinophilopoietic lymphokines and identity with interleukin-5. *Eur J Biochem*. 1988; 174:345–52. [PubMed: 3133208]

27. Campbell HD, et al. Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin 5). *Proc Natl Acad Sci U S A*. 1987; 84:6629–33. [PubMed: 3498940]
28. Handman E, Burgess AW. Stimulation by granulocyte-macrophage colony-stimulating factor of *Leishmania tropica* killing by macrophages. *J Immunol*. 1979; 122:1134–7. [PubMed: 312818]
29. Hamilton JA, Stanley ER, Burgess AW, Shadduck RK. Stimulation of macrophage plasminogen activator activity by colony-stimulating factors. *J Cell Physiol*. 1980; 103:435–45. [PubMed: 6967488]
30. Begley CG, et al. Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils in vitro: a rapid and sensitive microassay for colony-stimulating factors. *Blood*. 1986; 68:162–6. [PubMed: 3487354]
31. Dessein AJ, Vadas MA, Nicola NA, Metcalf D, David JR. Enhancement of human blood eosinophil cytotoxicity by semi-purified eosinophil colony-stimulating factor(s). *J Exp Med*. 1982; 156:90–103. [PubMed: 6979609]
32. Lopez AF, et al. Murine eosinophil differentiation factor. An eosinophil-specific colony-stimulating factor with activity for human cells. *J Exp Med*. 1986; 163:1085–99. [PubMed: 3486243]
33. Lopez AF, et al. Activation of granulocyte cytotoxic function by purified mouse colony-stimulating factors. *J Immunol*. 1983; 131:2983–8. [PubMed: 6605995]
34. Vadas MA, Lopez AF. Regulation of granulocyte function by colony stimulating factors. *Lymphokine Res*. 1984; 3:45–50. [PubMed: 6611465]
35. Vadas MA, Nicola NA, Metcalf D. Activation of antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. *J Immunol*. 1983; 130:795–9. [PubMed: 6600255]
36. Lieschke GJ, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood*. 1994; 84:1737–46. [PubMed: 7521686]
37. Lieschke GJ, et al. Mice lacking both macrophage- and granulocyte-macrophage colony-stimulating factor have macrophages and coexistent osteopetrosis and severe lung disease. *Blood*. 1994; 84:27–35. [PubMed: 8018921]
38. Stanley E, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci U S A*. 1994; 91:5592–6. [PubMed: 8202532]
39. Metcalf D, Begley CG, Nicola NA, Johnson GR. Quantitative responsiveness of murine hemopoietic populations in vitro and in vivo to recombinant multi-CSF (IL-3). *Exp Hematol*. 1987; 15:288–95. [PubMed: 3493174]
40. Metcalf D, et al. Hemopoietic responses in mice injected with purified recombinant murine GM-CSF. *Exp Hematol*. 1987; 15:1–9. [PubMed: 3490993]
41. Lang RA, et al. Transgenic mice expressing a hemopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. *Cell*. 1987; 51:675–86. [PubMed: 3499986]
42. Campbell IK, et al. Protection from collagen-induced arthritis in granulocyte-macrophage colony-stimulating factor-deficient mice. *J Immunol*. 1998; 161:3639–44. [PubMed: 9759887]
43. Cook AD, et al. Granulocyte-macrophage colony-stimulating factor is a key mediator in inflammatory and arthritic pain. *Ann Rheum Dis*. 2012
44. Cook AD, et al. Granulocyte-macrophage colony-stimulating factor is a key mediator in experimental osteoarthritis pain and disease development. *Arthritis Res Ther*. 2012; 14:R199. [PubMed: 22995428]
45. Lawlor KE, et al. Critical role for granulocyte colony-stimulating factor in inflammatory arthritis. *Proc Natl Acad Sci U S A*. 2004; 101:11398–403. [PubMed: 15272075]
46. Morstyn G, et al. Effect of granulocyte colony stimulating factor on neutropenia induced by cytotoxic chemotherapy. *Lancet*. 1988; 1:667–72. [PubMed: 2895212]
47. Dührsen U, et al. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood*. 1988; 72:2074–81. [PubMed: 3264199]

48. Juttner CA, To LB, Haylock DN, Branford A, Kimber RJ. Circulating autologous stem cells collected in very early remission from acute non-lymphoblastic leukaemia produce prompt but incomplete haemopoietic reconstitution after high dose melphalan or supralesional chemoradiotherapy. *Br J Haematol.* 1985; 61:739–45. [PubMed: 2867773]
49. Sheridan WP, et al. Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. *Lancet.* 1992; 339:640–4. [PubMed: 1371817]
50. Lieschke GJ, et al. Effects of bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor in patients with advanced malignancy. *Ann Intern Med.* 1989; 110:357–64. [PubMed: 2644886]
51. Villeval JL, Duhrsen U, Morstyn G, Metcalf D. Effect of recombinant human granulocyte-macrophage colony stimulating factor on progenitor cells in patients with advanced malignancies. *Br J Haematol.* 1990; 74:36–44. [PubMed: 2178672]
52. Nicola NA, Metcalf D. Binding of the differentiation-inducer, granulocyte-colony-stimulating factor, to responsive but not unresponsive leukemic cell lines. *Proc Natl Acad Sci U S A.* 1984; 81:3765–9. [PubMed: 6610179]
53. Nicola NA, Metcalf D. Binding of 125I-labeled granulocyte colony-stimulating factor to normal murine hemopoietic cells. *J Cell Physiol.* 1985; 124:313–21. [PubMed: 3876343]
54. Nicola NA, Metcalf D. Binding of iodinated multipotential colony-stimulating factor (interleukin-3) to murine bone marrow cells. *J Cell Physiol.* 1986; 128:180–8. [PubMed: 3090054]
55. Walker F, Burgess AW. Specific binding of radioiodinated granulocyte-macrophage colony-stimulating factor to hemopoietic cells. *EMBO J.* 1985; 4:933–9. [PubMed: 2990915]
56. Walker F, Nicola NA, Metcalf D, Burgess AW. Hierarchical down-modulation of hemopoietic growth factor receptors. *Cell.* 1985; 43:269–76. [PubMed: 3000602]
57. Gearing DP, King JA, Gough NM, Nicola NA. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. *EMBO J.* 1989; 8:3667–76. [PubMed: 2555171]
58. Hilton DJ, et al. Cloning of a murine IL-11 receptor alpha-chain; requirement for gp130 for high affinity binding and signal transduction. *EMBO J.* 1994; 13:4765–75. [PubMed: 7957045]
59. Hilton DJ, et al. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc Natl Acad Sci U S A.* 1996; 93:497–501. [PubMed: 8552669]
60. Hayashida K, et al. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. *Proc Natl Acad Sci U S A.* 1990; 87:9655–9. [PubMed: 1702217]
61. Lopez AF, et al. Residue 21 of human granulocyte-macrophage colony-stimulating factor is critical for biological activity and for high but not low affinity binding. *EMBO J.* 1992; 11:909–16. [PubMed: 1532144]
62. Sun Q, et al. Simultaneous antagonism of interleukin-5, granulocyte-macrophage colony-stimulating factor, and interleukin-3 stimulation of human eosinophils by targeting the common cytokine binding site of their receptors. *Blood.* 1999; 94:1943–51. [PubMed: 10477723]
63. Hansen G, et al. The structure of the GM-CSF receptor complex reveals a distinct mode of cytokine receptor activation. *Cell.* 2008; 134:496–507. [PubMed: 18692472]
64. Leung DW, et al. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature.* 1987; 330:537–43. [PubMed: 2825030]
65. Brown RJ, et al. Model for growth hormone receptor activation based on subunit rotation within a receptor dimer. *Nat Struct Mol Biol.* 2005; 12:814–21. [PubMed: 16116438]
66. Varghese JN, et al. Structure of the extracellular domains of the human interleukin-6 receptor alpha-chain. *Proc Natl Acad Sci U S A.* 2002; 99:15959–64. [PubMed: 12461182]
67. Huyton T, et al. An unusual cytokine:Ig-domain interaction revealed in the crystal structure of leukemia inhibitory factor (LIF) in complex with the LIF receptor. *Proc Natl Acad Sci U S A.* 2007; 104:12737–42. [PubMed: 17652170]
68. Xu Y, et al. Crystal structure of the entire ectodomain of gp130: insights into the molecular assembly of the tall cytokine receptor complexes. *J Biol Chem.* 2010; 285:21214–8. [PubMed: 20489211]

69. Gearing DP, et al. Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). *EMBO J.* 1987; 6:3995–4002. [PubMed: 3127201]
70. Williams RL, et al. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature.* 1988; 336:684–7. [PubMed: 3143916]
71. Van Snick J, et al. cDNA cloning of murine interleukin-HP1: homology with human interleukin 6. *Eur J Immunol.* 1988; 18:193–7. [PubMed: 2965020]
72. Van Snick J, et al. Purification and NH₂-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas. *Proc Natl Acad Sci U S A.* 1986; 83:9679–83. [PubMed: 2948184]
73. Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci U S A.* 1985; 82:8667–71. [PubMed: 3866246]
74. Gamble JR, Vadas MA. Endothelial adhesiveness for blood neutrophils is inhibited by transforming growth factor-beta. *Science.* 1988; 242:97–9. [PubMed: 3175638]
75. Xia P, et al. Sphingosine kinase interacts with TRAF2 and dissects tumor necrosis factor-alpha signaling. *J Biol Chem.* 2002; 277:7996–8003. [PubMed: 11777919]
76. Groom J, et al. Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. *J Clin Invest.* 2002; 109:59–68. [PubMed: 11781351]
77. Thien M, et al. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity.* 2004; 20:785–98. [PubMed: 15189742]
78. Lacy P, Stow JL. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. *Blood.* 2011; 118:9–18. [PubMed: 21562044]
79. Murray RZ, Kay JG, Sangermani DG, Stow JL. A role for the phagosome in cytokine secretion. *Science.* 2005; 310:1492–5. [PubMed: 16282525]
80. Wilks AF. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. *Proc Natl Acad Sci U S A.* 1989; 86:1603–7. [PubMed: 2466296]
81. Wilks AF, et al. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. *Mol Cell Biol.* 1991; 11:2057–65. [PubMed: 1848670]
82. Nicholson SE, et al. Tyrosine kinase JAK1 is associated with the granulocyte-colony-stimulating factor receptor and both become tyrosine-phosphorylated after receptor activation. *Proc Natl Acad Sci U S A.* 1994; 91:2985–8. [PubMed: 7512720]
83. Lucet IS, et al. The structural basis of Janus kinase 2 inhibition by a potent and specific pan-Janus kinase inhibitor. *Blood.* 2006; 107:176–83. [PubMed: 16174768]
84. Starr R, et al. A family of cytokine-inducible inhibitors of signalling. *Nature.* 1997; 387:917–21. [PubMed: 9202125]
85. Hilton DJ, et al. Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc Natl Acad Sci U S A.* 1998; 95:114–9. [PubMed: 9419338]
86. Zhang JG, et al. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. *Proc Natl Acad Sci U S A.* 1999; 96:2071–6. [PubMed: 10051596]
87. Nicholson SE, et al. Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. *Proc Natl Acad Sci U S A.* 2000; 97:6493–8. [PubMed: 10829066]
88. Nicholson SE, et al. Mutational analyses of the SOCS proteins suggest a dual domain requirement but distinct mechanisms for inhibition of LIF and IL-6 signal transduction. *EMBO J.* 1999; 18:375–85. [PubMed: 9889194]
89. Croker BA, et al. SOCS3 negatively regulates IL-6 signaling in vivo. *Nat Immunol.* 2003; 4:540–5. [PubMed: 12754505]
90. Croker BA, et al. SOCS3 is a critical physiological negative regulator of G-CSF signaling and emergency granulopoiesis. *Immunity.* 2004; 20:153–65. [PubMed: 14975238]

91. Metcalf D, et al. Gigantism in mice lacking suppressor of cytokine signalling-2. *Nature*. 2000; 405:1069–73. [PubMed: 10890450]
92. Alexander WS, et al. SOCS1 is a critical inhibitor of interferon gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell*. 1999; 98:597–608. [PubMed: 10490099]
93. Babon JJ, et al. Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. *Immunity*. 2012; 36:239–50. [PubMed: 22342841]
94. Tebbutt NC, et al. Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. *Nat Med*. 2002; 8:1089–97. [PubMed: 12219085]
95. Ernst M, Jenkins BJ. Acquiring signalling specificity from the cytokine receptor gp130. *Trends Genet*. 2004; 20:23–32. [PubMed: 14698616]
96. Langdon WY, Hartley JW, Klinken SP, Ruscetti SK, Morse HC 3rd. Molecular characterization of a transforming retrovirus involved in pre-B cell lymphomas. *Curr Top Microbiol Immunol*. 1988; 141:27–30. [PubMed: 2850894]
97. Thien CB, Langdon WY. Cbl: many adaptations to regulate protein tyrosine kinases. *Nat Rev Mol Cell Biol*. 2001; 2:294–307. [PubMed: 11283727]
98. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature*. 1988; 335:440–2. [PubMed: 3262202]
99. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol*. 2008; 9:47–59. [PubMed: 18097445]
100. Guthridge MA, et al. Site-specific serine phosphorylation of the IL-3 receptor is required for hemopoietic cell survival. *Mol Cell*. 2000; 6:99–108. [PubMed: 10949031]
101. Bowtell D, Fu P, Simon M, Senior P. Identification of murine homologues of the *Drosophila* son of sevenless gene: potential activators of ras. *Proc Natl Acad Sci U S A*. 1992; 89:6511–5. [PubMed: 1631150]
102. Rozakis-Adcock M, Fernley R, Wade J, Pawson T, Bowtell D. The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1. *Nature*. 1993; 363:83–5. [PubMed: 8479540]
103. Daly RJ, Sanderson GM, Janes PW, Sutherland RL. Cloning and characterization of GRB14, a novel member of the GRB7 gene family. *J Biol Chem*. 1996; 271:12502–10. [PubMed: 8647858]
104. Cooney GJ, et al. Improved glucose homeostasis and enhanced insulin signalling in Grb14-deficient mice. *EMBO J*. 2004; 23:582–93. [PubMed: 14749734]
105. Brummer T, et al. Phosphorylation-dependent binding of 14-3-3 terminates signalling by the Gab2 docking protein. *EMBO J*. 2008; 27:2305–16. [PubMed: 19172738]
106. Isaacs A, Edney M. Interference between inactive and active influenza viruses in the chick embryo. I. Quantitative aspects of interference. *Aust J Exp Biol Med Sci*. 1950; 28:219–30. [PubMed: 14772162]
107. Isaacs A, Lindenmann J. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci*. 1957; 147:258–67. [PubMed: 13465720]
108. Sadler AJ, Williams BR. Structure and function of the protein kinase R. *Curr Top Microbiol Immunol*. 2007; 316:253–92. [PubMed: 17969452]
109. Irving AT, et al. Regulation of actin dynamics by protein kinase R control of gelsolin enforces basal innate immune defense. *Immunity*. 2012; 36:795–806. [PubMed: 22633459]
110. Hwang SY, et al. A null mutation in the gene encoding a type I interferon receptor component eliminates antiproliferative and antiviral responses to interferons alpha and beta and alters macrophage responses. *Proc Natl Acad Sci U S A*. 1995; 92:11284–8. [PubMed: 7479980]
111. Piganis RA, et al. Suppressor of cytokine signaling (SOCS) 1 inhibits type I interferon (IFN) signaling via the interferon alpha receptor (IFNAR1)-associated tyrosine kinase Tyk2. *J Biol Chem*. 2011; 286:33811–8. [PubMed: 21757742]
112. Mansell A, Brint E, Gould JA, O'Neill LA, Hertzog PJ. Mal interacts with tumor necrosis factor receptor-associated factor (TRAF)-6 to mediate NF-kappaB activation by toll-like receptor (TLR)-2 and TLR4. *J Biol Chem*. 2004; 279:37227–30. [PubMed: 15247281]

113. Mansell A, et al. Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nat Immunol.* 2006; 7:148–55. [PubMed: 16415872]
114. Whitmore MM, et al. Negative regulation of TLR-signaling pathways by activating transcription factor-3. *J Immunol.* 2007; 179:3622–30. [PubMed: 17785797]
115. Hart PH, et al. Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. *Proc Natl Acad Sci U S A.* 1989; 86:3803–7. [PubMed: 2786204]
116. Martin JL, Baxter RC. Insulin-like growth factor-binding protein from human plasma. Purification and characterization. *J Biol Chem.* 1986; 261:8754–60. [PubMed: 3722172]
117. Wood WI, et al. Cloning and expression of the growth hormone-dependent insulin-like growth factor-binding protein. *Mol Endocrinol.* 1988; 2:1176–85. [PubMed: 2464130]
118. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev.* 2002; 23:824–54. [PubMed: 12466191]
119. Garrett TP, et al. Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. *Nature.* 1998; 394:395–9. [PubMed: 9690478]
120. McKern NM, et al. Structure of the insulin receptor ectodomain reveals a folded-over conformation. *Nature.* 2006; 443:218–21. [PubMed: 16957736]
121. Denley A, Cosgrove LJ, Booker GW, Wallace JC, Forbes BE. Molecular interactions of the IGF system. *Cytokine Growth Factor Rev.* 2005; 16:421–39. [PubMed: 15936977]
122. Ross M, Francis GL, Szabo L, Wallace JC, Ballard FJ. Insulin-like growth factor (IGF)-binding proteins inhibit the biological activities of IGF-1 and IGF-2 but not des-(1-3)-IGF-1. *Biochem J.* 1989; 258:267–72. [PubMed: 2539101]
123. Garrett TP, et al. Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. *Cell.* 2002; 110:763–73. [PubMed: 12297049]
124. Burgess AW, et al. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. *Mol Cell.* 2003; 12:541–52. [PubMed: 14527402]
125. Garrett TP, et al. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol Cell.* 2003; 11:495–505. [PubMed: 12620236]
126. Alon T, et al. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med.* 1995; 1:1024–8. [PubMed: 7489357]
127. Stone J, et al. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. *J Neurosci.* 1995; 15:4738–47. [PubMed: 7623107]
128. Achen MG, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci U S A.* 1998; 95:548–53. [PubMed: 9435229]
129. Stacker SA, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med.* 2001; 7:186–91. [PubMed: 11175849]
130. Baldwin ME, et al. Vascular endothelial growth factor D is dispensable for development of the lymphatic system. *Mol Cell Biol.* 2005; 25:2441–9. [PubMed: 15743836]