Acta Biochimica Polonica

Vol. 50 No. 3/2003

583–594

QUARTERLY

Review

Plasma membrane rafts and chaperones in cytokine/STAT signaling★✪

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Received: 30 May, 2003; revised: 21 July, 2003; accepted: 19 August, 2003

Key words: signal transducers and activators of transciption (STATs), cytokine receptors, lipid rafts, caveolin, chaperones, heat shock protein 90 (HSP90), fever

We and others have recently obtained data suggesting that cytokine-STAT signaling in many different cell-types is a chaperoned pathway initiated at the level of specialized plasma membrane microdomains called "rafts" (the "raft-STAT signaling hypothesis"). These findings are of broad significance in that all cytokines and growth factors initiate signaling in target cells by interacting with respective cell-surface receptors. The new data suggest that raft microdomains represent the units of function at the cell-surface through which ligand-stimulated STAT signaling is initiated. Moreover, recent evidence shows the involvement of chaperone proteins in regulating the STAT signaling pathway. These chaperones include the human homolog of the tumorous imaginal disc 1 protein (hTid1) which associates with Janus kinase 2 (JAK2) at the level of the plasma membrane, heat shock protein 90 (HSP90) which associates with STAT3 and STAT1 proteins in caveolin-1-containing raft and cytoplas-

★Presented at the XXX Winter School of Faculty of Biotechnology, Jagiellonian University, Kościelisko, Poland, 28th February–4th March, 2003.

✪Supported in part by Research Grant HL073301 from The National Institutes of Health, U.S.A.

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Abbreviations: cav, caveolin; CRP, C-reactive protein; EGF, epidermal growth factor; eNOS, endothelial cell-specific nitric oxide synthase; GA, geldanamycin; gp130, the gp130 signal transducing chain in the IL-6 receptor complex; GRP58, glucose regulated protein 58 (also abbreviated ER-60 and ERp57); HSP90, heat shock protein 90; IFN, interferon; IL, interleukin; JAK, Janus kinase; MCD, methyl-β-cyclodextrin; MCT, monocrotaline; PDGF, platelet-derived growth factor; PH, pulmonary hypertension; STAT, Signal Transducers and Activators of Transciption protein family; Tid1, tumorous imaginal disc 1 protein; TNF-α, tumor necrosis factor α.

mic complexes, and glucose regulated protein 58 (GRP58/ER-60/ERp57), a thiol dependent protein-disulfide isomerase, found in association with STAT3 "statosome" complexes in the cytosol and in the raft fraction. We suggest a function of the HSP90 chaperone system in preserving IL-6/STAT3 signaling in liver cells in the context of fever. The identification and function of protein partners associated with specific STAT species in rafts and in cytosolic complexes, and in the efficient departure of cytokine-activated STATs from the cytosolic face of rafts towards the cell nucleus are now areas of active investigation.

We and others have discovered that functional cytokine-stimulated STAT transcription factor signaling is a chaperoned pathway initiated at the level of the cholesterol-rich plasma membrane "raft" microdomain (Fig. 1). Today's insights into raft-STAT signaling and the role of chaperones which preserve this signaling pathway under temperature stress (such as during fever) emanate from two specific lines of research – one begun over 70 years ago in the area of clinical medicine, and the other begun over 30 years ago in the area of animal virology. In order to put the new insights in the area of rafts and chaperones in cytokine-STAT signaling into proper perspective we shall first briefly review this earlier literature, and then show how modern-day insights into cytokine/raft/STAT signaling so elegantly relate back to the older clinical and biological observations.

THE ACUTE-PHASE RESPONSE

In a seminal paper, published in 1930, Tillet and Francis pointed out that patients with pneumococcal pneumonia upon admission to a hospital displayed high fever which was sustained for only few days (2–4 days), and then rapidly returned to baseline values (Tillet & Francis, 1930). These investigators collected serum from such patients during the pyrexic

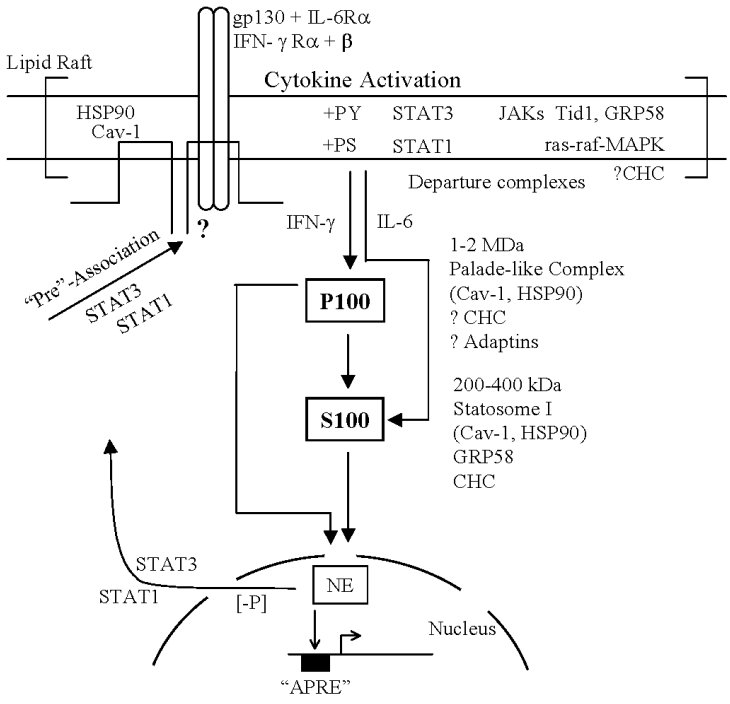

Figure 1. The raft-STAT signaling hypothesis and chaperoned transcytoplasmic trafficking of STATs.

Cytoplasmic fractions designated P100 and S100 correspond respectively to the 100 000 × ***g*** sedimentable and soluble fractions (Ndubuisi *et al.*, 1999).

acute phase as well as during the later convalescent phase. Sera from patients in the acute phase already at admission contained very high titers of a material which gave a precipitin reaction with the somatic "C" polysaccharide fraction of peumococcus. In contrast, the agglutination titer in serum to a type-specific antigen of pneumococcus was low upon admission but gradually increased several days later. While the latter delayed response was readily understood to be due to the stimulation of specific anti-pneumococcal antibodies, the rapid and early high titers of fraction C-reactive precipitin in serum were difficult to explain. Moreover, patients with *other* bacterial infections accompanied by fever also displayed high serum titers of a precipitin reactive with the *pneumococcal* fraction C. The interpretation of these data at that time was in terms of the then newly recognized phenomenon of an anamanestic response. Tillet and Francis (1930) suggested that the rapid increase in serum titers of a precipitin reactive with the penumococcal fraction C in sera of patients with acute fever (with pneumococcal pneumonia or with infections caused by other bacteria) might be due to an anamanestic response by the host (i.e. the rapid stimulation by an antigen of the production of antibodies to unrelated antigens). As happens often in science, the experimental observations have stood the test of time, while the particular interpretation has been replaced by an alternative one. We now know that the fraction C-reactive precipitin in sera of patients acutely ill with a variety of infections was not an antibody at all but a host protein now called the C-reactive protein (CRP) (reviewed in Koj, 1974; Kushner *et al.*, 1982). The marked increases in serum CRP levels and a whole constellation of other plasma proteins is a hallmark of the "acute phase response" (reviewed in Koj, 1974; Kushner *et al.*, 1982; Sehgal *et al.*, 1989).

Through the second half of the twentieth century, CRP was purified and characterized, it was shown that CRP was synthesized in the liver, and that the increased production of CRP by the hepatocyte during acute infection and fever was the result of the release of a blood-borne mediator protein which travelled from the site of infection to the liver and upregulated CRP gene transcription ("the hepatocyte stimulating factor") (reviewed in Kushner *et al.*, 1982). The major systemic regulator of CRP during the acute phase response was identified to be the cytokine interleukin-6 (IL-6) (Gauldie *et al.*, 1987; reviewed in Sehgal *et al.*, 1989) and the major transcription factor involved in upregulating the CRP gene promoter was identified to be STAT3 (reviewed in Mackiewicz *et al.*, 1994). Shortly after the recognition of IL-6 as the hepatocyte stimulating factor, Aarden and colleagues in 1987 confirmed the relationship between elevated circulating levels of IL-6, elevated body temperature and the elevation of serum CRP levels (Nijsten *et al.*, 1987). IL-6 is now known to be a potent central pyrogen (reviewed in Leon, 2002). These medical and biological observations present us with two questions. First, how is IL-6/STAT signaling initiated at the cell surface? Second, how is IL-6/STAT3 signaling maintained in hepatocytes in the face of elevated body temperature? New data obtained in recent years show that cytokine receptors are localized in specialized cholesterol-rich detergent-resistant microdomains of the plasma membrane called "rafts". These raft microdomains appear to represent the unit of function through which cytokine signaling is initiated (the raft-STAT signaling hypothesis). Moreover, recent data show the involvement of the chaperone heat shock protein 90 (HSP90) in preserving IL-6 signaling in liver cells during fever (Shah *et al.*, 2002).

LIPID RAFTS AND CAVEOLAE IN CELL SIGNALING

Lipid raft microdomains as understood today consist of dynamic assemblies of choles-

terol and sphingolipids in the plane of the plasma membrane bilayer (Simons & Toomre, 2000; Galbiati *et al.*, 2001; Anderson *et al.*, 2002; Simons & Ehehalt, 2002). It is now widely accepted that lipid rafts organize the plasma membrane into functional units. Raft microdomains represent collecting sites on the cell surface for multiple glycophosphatidyl (GPI)-anchored membrane proteins, and for proteins targeted to rafts through binding to integral raft proteins or through non-covalently bound "lipid shells". These raft domains can then act as platforms for conducting a variety of cellular functions, including signal transduction (Fig. 1).

It is worth taking another detour through history in order to understand the origins of the lipid raft concept and of many of the techniques used today to investigate raft structure. These origins lie in the studies of animal virologists over 30 years ago who investigated the maturation and budding of enveloped RNA viruses from the plasma membrane. As examples, immunoelectronmicroscopic studies of cells infected with the parainfluenza virus Simian Virus 5 showed the insertion of viral glycoproteins into "localized patches in the plasma membrane" (Compans & Choppin, 1973). In subsequent steps of the maturation process viral nucleocapsid proteins were observed to be apposed to the cytoplasmic face of such patches followed by assembly of the nucleocapsid structure and eventual viral budding. Choppin and colleagues analysed the lipid composition of virus-associated plasma membrane and compared it to that of the plasma membrane of the host cell (Klenk & Choppin, 1970a; 1970b). These investigators observed that, while the overall composition of viral lipids reflected that of the plasma membrane of the host cell from which the virus was derived, there was a relative enrichment of cholesterol and sphingolipids in the virus-associated plasma membrane. Today we recognize that lipid raft microdomains represent cholesterol- and sphingolipid-enriched dynamic patches in the plasma membrane (Simons & Toomre, 2000; Galbiati *et al.*, 2001; Anderson & Jacobson, 2002; Simons & Ehehalt, 2002).

Beginning with the observations of Sabatini and colleagues in the late 1970s it is now established that specific enveloped viruses mature and bud from cells in a polarized manner (Rodriquez-Boulan & Sabatini, 1978). As examples, influenza virions mature on the apical side of polarized epithelial cells, while vesicular stomatis virions mature from the basolateral side. In studying how viral glycoproteins were selectively inserted into respective plasma membrane microdomains, virologists developed the technique of isolating the plasma membrane fraction, then treating it with non-ionic detergents followed by equilibrium density flotation centrifugation. The cholesterol-rich plasma membrane "raft" which resisted solubilization by non-ionic detergents (such as Triton X-100) in the cold (at 4°C) had a lower bouyant density and floated to the top carrying with it virus-specific glycoprotein complexes (Kurzchalia *et al.*, 1992). In 1992, Simons and colleagues extended such flotation assays using uninfected cells and discovered that normal cells also yielded a flotation raft fraction (Kurzchalia *et al.*, 1992). Upon characterizing the major proteins in such flotation rafts derived from uninfected cells, these investigators identified a major protein of 21 kDa (VIP21). An antibody to VIP21 showed that this protein was localized to plasma membrane invaginations that had been discovered previously and called "caveolae". Independently, the same protein was identified as the major cellular substrate for Tyr-phosphorylation by v-src in chicken cells (Rothberg *et al.*, 1992). Again, an antibody to this protein localized it to caveolae. This molecule was thus called caveolin (now caveolin-1 or cav-1) (Rothberg *et al.*, 1992) and represents the first "integral" raft protein to be identified. When cav-1 is inserted into a lipid raft from the cytoplasmic surface, the protein self-stacks and oligomerizes ($n \geq 15$) through its cav-1 stacking domain into a

supramolecular assembly which results in flask-shaped invaginations of the plasma membrane of diameter 50–100 nm (the "caveolae") (Okamoto *et al.*, 1998) (Fig. 1). Integral/structural raft proteins now known include caveolin-1, 2, 3, flotillin-1, 2, and stomatins (reviewed in Galbiati *et al.*, 2001). It is now recognized that not all plasma membrane lipid rafts contain cav-1, nor are all lipid rafts restricted to caveolar structures. In a validation of the older virological literature, recent studies have shown that the maturation of an enveloped virus, such as respiratory syncytial virus, from the cell surface is preceded by the co-localization of cav-1 and viral glycoproteins into the same plasma membrane rafts and that cellular cav-1 is found in the mature virion (Brown *et al.*, 2002).

Caveolae, and more generally lipid rafts, have now been implicated in signal transduction (see Simons & Toomre, 2000, and Simons & Ehehalt, 2002). Biochemical and morphological experiments have shown that a variety of signaling molecules are concentrated within these plasma membrane microdomains, such as Src family tyrosine kinases, Ha-Ras, endothelial nitric oxide synthase (eNOS), and heterotrimeric G proteins (the "caveola signaling hypothesis"; Lisanti *et al.*, 1994; Li *et al.*, 1996). Signaling processes initiated in lipid rafts include immunoglobulin E signaling, T-cell antigen receptor signaling, B-cell receptor signaling, and signaling involving angiotensin II receptor, epidermal growth factor (EGF) receptor, platelet derived growth factor (PDGF) receptor, insulin receptor, EphrinB1 receptor, neurotrophin, Hedgehog, Ha-Ras, eNOS and integrins (reviewed in Simons & Toomre, 2002; Galbiati *et al.*, 2001; Anderson & Jacobson, 2002; Simons & Ehehalt, 2002).

Inhibitors of lipid raft function include agents such as filipin III and methyl-β-cyclodextrin (MCD) which extract cholesterol out of the cholesterol-rich rafts and peptide inhibitors corresponding to the cav-1 self-stacking domain and cav-1 binding domains from various target proteins (Simons & Toomre, 2000; Galbiati *et al.*, 2001). The resulting modulation of signaling is cell-type and ligand-dependent in that in some systems agents such as filipin III and MCD inhibit signaling, whereas in others disruption of raft function enhances signaling (see Muller *et al.*, 2001, and references cited therein).

The incorporation of cav-1 oligomers into the cytosolic face of plasma membrane domains is only one mechanism to form and stabilize lipid raft microdomains. The plasma membrane of hematopoietic lineage cells, which usually express little cav-1, is also organized into detergent-resistant raft microdomains (Fra *et al.*, 1994) which can function in the context of T and B cell signaling, and even cytokine signaling (see below).

INITIATION OF CYTOKINE-STIMULATED STAT SIGNALING IN CAV-1-CONTAINING, CHOLESTEROL-RICH PLASMA MEMBRANE RAFTS (THE "RAFT-STAT SIGNALING" HYPOTHESIS)

Recent data from this and several other laboratories show the localization of the JAK kinases 1 and 2 and Tyk2, the transcription factors STAT1 and STAT3 as well as activated PY-STAT1 and PY-STAT3, and the cytokine receptor chains for IL-6, IL-2, interferon-γ (IFN-γ), IFN-α/β and tumor necrosis factor-α (TNF-α) (gp130, IFN-γR1 and IFN-γR2, IL-2Rα, IFNAR1, IFNAR2, and the TNF-αR CD120a chain respectively) in detergent-resistant cav-1-containing plasma membrane raft fractions (see citations in Table 1). Table 1 and Fig. 1 summarize the new studies implicating rafts in cytokine/STAT signaling and the observations reported.

From a functional standpoint, we showed that the raft disrupters MCD markedly inhibited IL-6/STAT3 and IFN-γ/STAT1 signaling suggesting that almost all signaling by these cytokines was initiated in cholesterol-rich raft

Table 1. Recent studies reporting raft-STAT signaling

Study	Main findings
Koshelnick *et al.* (1997)	STAT1 and JAK1 observed in low-density detergent-resistant fraction of human kidney tumor epithelial cells (Line TCL-598) co-associated with cav-1 and the urokinase receptor. Clustering of urokinase receptor activated raft-STAT1 signaling.
Ju *et al.* (2000)	STAT3, Tyk2 and cav-1 co-associated in flotation rafts prepared from bovine aortic endothelial cells (BPAEC). Bradykinin activated STAT3 signaling in rafts.
Takaoka *et al.* (2000)	STAT1, JAK1, JAK2, both chains of each of the IFN-α and -γ receptors localized to cav-1-containing plasma membrane rafts in mouse fibroblasts. Filipin III reversibly inhibited IFN-γ/STAT1 signaling.
Martens *et al.* (2000)	gp130 localized to the basolateral but not apical side of polarized epithelial cells in culture.
Sadir *et al.* (2001)	Co-localization of IFN-γR1 chain with cav-1-containing caveolae and clathrin-coated pits in human U937 monocytic cells by immunoelectronmicroscopy.
Marmor & Julius (2001)	Localization of the IL-2Rα receptor chain in rafts in a mouse T cell line.
Sehgal *et al.* (2002)	Localization of gp130 and IFN-γR1 chains, STAT1, STAT3, PY-STAT1, PY-STAT3 in cav-1 and flotillin-1 containing rafts in human hepatoma Hep3B cells. Marked inhibition of IL-6 and IFN-γ signaling by MCD.
Cottin *et al.* (2002)	Localization of TNF receptor chain CD120a in rafts.
Shah *et al.* (2002)	Physical association betweeen STAT3, STAT1, cav-1 and HSP90 in the same detergent resistant unit in rafts, and in cytosolic complexes. Inhibition of IL-6 signaling by MCD, and elucidation of the function of HSP90 in the preservation of raft/IL-6/STAT3.
Bild *et al.* (2002)	Involvement of adaptin-containing complexes in growth factor/STAT3 signaling.
Subramaniam & Johnson (2002)	Localization of respective IFN-γR chains in rafts derived from cav-1-positive WISH cells and cav-1-negative Jurkat T cells. Inhibition of IFN-γ/STAT1 signaling in both cell types by MCD.
Podar *et al.* (2003)	Physical association between cav-1 and gp130 (and additional protein partners) in extracts derived from human myeloma cell lines; inhibition of IL-6 signaling by MCD.

microdomains (Sehgal *et al.*, 2002; Shah *et al.*, 2002). Moreover, Taniguchi and colleagues (Takaoka *et al.*, 2000) and Subramaniam & Johnson (2002) reported that the raft disrupter filipin III inhibited IFN-γ signaling in a reversible manner. The data of Subramaniam & Johnson (2002), derived from the cav-1-free Jurkat T cells, highlight the function of lipid rafts in cytokine signaling independent of the presence of cav-1. Clearly, there is now increasing evidence suggesting that substantial if not all cytokine/STAT signaling is initiated at the level of raft microdomains in diverse cell-types (the "raft-STAT signaling hypothesis") (Sehgal *et al.*, 2002).

CHAPERONES INVOLVED IN CYTOKINE-JAK-STAT SIGNALING

Chaperone proteins regulate the efficiency of biochemical and cellular processes, and protect the ongoing integrity of such pro-

cesses in the face of stress. Recent data from several laboratories show that cytokine/JAK/STAT signaling is a chaperoned pathway (Table 2 and Fig. 1). Pestka and colleagues used a yeast two-hybrid selection system to isolate proteins which interacted with JAK2. Among the proteins they identified was a DnaJ-like chaperone called Tid1 (Sarkar *et al.*, 2001). Human Tid1 (hTid1) is a chaperone which ordinarily mediates the import of proteins into mitochondria. However, Pestka and colleagues provided clear and convincing evidence for the presence of hTid1 in the cytosol and in the plasma membrane, its interaction with JAK2 in unstimulated cells, and the departure of Tid1 from JAK2-containing membrane complexes upon treatment of cells with IFN-γ. These authors suggested a "sequestration" model for the action of Tid1 in that this chaperone sequestered Jak2 in an inactive complex with the IFN-γ receptor at the level of the plasma membrane, and cytokine stimulation released Jak2/the receptor chains from this sequestration. Overexpression of Tid1 inhibited functional IFN-γ/JAK/STAT1 signaling as assayed using STAT1-responsive reporter/luciferase constructs.

Table 2. Chaperones involved in cytokine/JAK/STAT signaling

Study	Main findings
Ndubuisi *et al.* (1999)	Identification of the thiol-dependent protein disulfide isomerase GRP58/ER-60/ERp57 as a cytosolic STAT3 associated protein
Sarkar *et al.* (2001)	Identification of hTid1 (a DnaJ-like chaperone) as a JAK2 associated protein at the level of the plasma membrane and elucidation of its sequestering/inhibitory effect on IFN-γ/STAT1 signaling.
Guo *et al.* (2002)	Association of GRP58 with STAT3 in cytosolic complexes and sequestring/inhibitory effect of rGRP58 on DNA-binding activity of Tyr-phosphorylated STAT3.
Shah *et al.* (2002)	Identification of HSP90 as a STAT3-interacting chaperone at the levels of the plasma membrane raft and in cytosolic complexes and evidence for HSP90 function of in preserving IL-6/STAT3 signaling at both normal body temperature and during fever.
Sato *et al.(*2003)	Mapping the protein domains which mediate the interaction between HSP90 and STAT3 and evidence for HSP90 function in IL-6/STAT3 signaling at normal body temperature.

We have identified the chaperone and disulfide protein-isomerase GRP58/ER-60/ERp57 as a STAT3-associated protein at the level of the plasma membrane raft and in cytosolic complexes (Ndubuisi *et al.*, 1999; Sehgal, 2000; 2001; Guo *et al.*, 2002). Excess recombinant GRP58 inhibited the DNA-binding competence of cytosolic PY-STAT3 (Guo *et al.*, 2002). In transient transfection experiments overexpression of GRP58 in Hep3B cells inhibited both the basal activity and the ability of IL-6 to activate STAT3-responsive promoter-luciferase reporter constructs (unpublished data). Thus, like Tid1, GRP58 may function to sequester activated PY-STAT3 in cytokine-stimulated cells.

The identification of HSP90 as a potential candidate STAT3-associated protein (Shah *et al.*, 2002) was intriguing in that it is already known that other raft-involved signaling molecules such as eNOS also interact physically and functionally with both cav-1 and HSP90 (reviewed in Fleming & Busse, 1999, and in

Kone, 2000). Using the immunomagnetic-bead panning technique we confirmed that HSP90 was cross-precipitated by anti-STAT3 pAb from the membrane raft and cytoplasmic fractions (Shah *et al.*, 2002). HSP90 was also included with STAT3 in complexes immunopanned using anti-cav-1 pAb. Moreover, anti-GRP58 pAb also crosspanned HSP90 together with STAT3 from cytosolic complexes. STAT1 was also included in these cross-immunopanned complexes from the membrane and cytoplasmic fractions. Taken together, our magnetic-bead immunopanning data indicated that STAT3, cav-1 and HSP90 interacted within detergent-resistant physical units in the membrane raft and cytoplasmic compartments (Shah *et al.*, 2002).

The HSP90 inhibitor geldanamycin (GA) was used to probe the function of this chaperone in STAT3 signaling (Shah *et al.*, 2002). The design of these experiments involved maintaining confluent Hep3B cultures at 37°C or exposing them to 39.5°C for 12–16 h to raise their levels of HSP90 (a temperature stress equivalent to a fever of 103°F), followed by an evaluation of the effects of a 15 min pretreatment with GA (typically at 20 μM) on various IL-6-induced STAT3-mediated responses at the two temperatures. Remarkably, despite the elevated temperature used, functional IL-6/STAT3 signaling was preserved at 39.5°C at a level approximately half that at 37°C. In Hep3B hepatocytes, IL-6-stimulated PY-STAT3 activation and trafficking to the nucleus was only minimally inhibited at 37°C by GA. However, this inhibitor markedly reduced PY-STAT3 activation and trafficking at 39.5°C suggesting the critical need for HSP90 in preserving IL-6/STAT3 signaling at the higher temperature. Immunopanning and Western blotting analyses data confirmed that GA disrupted the physical interaction between HSP90 and STAT3 in cells at both 37°C and 39.5°C (Shah *et al.*, 2002). These observations suggested that HSP90 played a critical role in preserving IL-6/STAT signaling particularly at the elevated temperature.

We also investigated the effect of GA on the IL-6-induced upregulation of a reporter/luciferase construct containing four copies of the STAT3-binding element (Shah *et al.*, 2002). The inducibility of this reporter construct by IL-6 was maintained at 39.5°C at a level approximately half of that at 37°C. While GA had a modest inhibitory effect on IL-6-inducibility of this reporter construct at 37°C (4-fold reduction), this inhibitor dramatically reduced reporter construct inducibility at 39.5°C (10-fold reduction). These data show that while HSP90 did contribute towards chaperoning IL-6/STAT3 signaling at both temperatures as evaluated in STAT3/luciferase reporter assays, there was a particularly dramatic role for HSP90 in preserving STAT3 signaling at 39.5°C (a temperature which corresponds to a fever of 103°F).

The involvement of HSP90 in IL-6/STAT3 signaling has now been confirmed independently (Sato *et al.*, 2003). In experiments carried out at 37°C, these investigators observed that GA modestly inhibited IL-6-induced STAT3/luciferase reporter activity (by 3–4-fold). Transfection of a constitutive overexpression construct for HSP90 into cells treated with GA reversed the ability of this inhibitor to reduce the level of IL-6-activated STAT3-luciferase activity. Moreover in cross-immunoprecipitation assays using cells transiently transfected with various deletion mutants of HSP90 and of STAT3, evidence was obtained suggesting that the N-terminal geldanamycin-binding domain in HSP90 interacted with the DNA-binding domain in STAT3 (Sato *et al.*, 2003).

To summarize, it has now become apparent that cytokine-raft-JAK-STAT signaling is a chaperoned pathway. Three of the chaperones involved are Tid1, GRP58 and HSP90 (Table 2). The role of HSP90 in preserving raft-STAT3 signaling in the context of fever brings us full circle to the observations of Tillet and Francis over 70 years ago. It is particularly satisfying that biochemical and molecular studies in this laboratory in recent

years have provided an explanation for how IL-6/STAT3 signaling continues to function during fever. Although IL-6 is pyrogenic and appears in the body during the "acute phase" at a time when there is elevation of body temperature, the HSP90 chaperone system provides an elegant mechanism to preserve IL-6/STAT3 signaling in hepatocytes during fever ensuring the sustained production of "protective" acute-phase plasma proteins.

A DISEASE MECHANISM INVOLVING CAV-1 RAFT DISRUPTION AND HYPERACTIVATION OF IL-6/STAT3 SIGNALING: PULMONARY HYPERTENSION IN THE MONOCROTALINE (MCT)-TREATED RAT

We have associated the disruption of cav-1 raft mechanisms and consequent hyperactivation of IL-6/PY-STAT3 signaling with a specific disease mechanism in an experimental animal model (Mathew *et al.*, 2003). Rats treated with a single injection of the plant alkaloid monocrotaline (MCT) develop progressive pulmonary hypertension (PH) 10–14 days later (reviewed in Shultze & Roth, 1998; Bhargawa *et al.*, 1999). It has been previously shown that the development of PH in this model is accompanied by upregulation of IL-6 production locally in the lung tissue (Bhargava *et al.*, 1999). It has also been shown previously that the liver converts inactive MCT into the active pyrrole moiety which damages the pulmonary arterial endothelium during its first pass through the lung within 48 h (Shultze & Roth, 1998). We observed a marked downregulation of cav-1 in lung extracts prepared from MCT-treated rats within 48 h accompanied by a subsequent increase in tissue levels of PY-STAT3 (Mathew *et al.*, 2003). A reduction in cav-1 mRNA, but not in cav-2 or cav-3 mRNA levels, also occurred in the lungs of MCT-treated rats by 7–14 days. It has been suggested that the initiating mechanism in this model of PH may be the disruption of cav-1 rafts by MCT-pyrrole followed by the dysregulation of cytokine and STAT signaling, an hyperproliferative response of the pulmonary endothelium, and vascular lumen blockage. Consistent with this proposal is the observation that *cav-1* (–/–) null mice develop marked pulmonary hypertension and right heart failure (Zhao *et al.*, 2002).

CONCLUDING COMMENTS

Our discovery of STATs in rafts was an extension of work characterizing the subcellular distribution of STAT proteins using conventional cell-fractionation techniques. The process of cytokine receptor/raft/STAT signaling is likely to be broad significance in that this model may apply to signaling by STATs in, perhaps, all cell-types. The functional insights into the role of HSP90-based chaperones in STAT3 signaling are also likely of broad relevance in that these may relate to the mechanism of action of most cytokines, particularly in the context of elevated body temperature. The new data suggest that the efficient departure of activated STATs from the cytosolic face of the plasma membrane raft towards the cell interior and subsequent transcytoplasmic trafficking involves specific protein partners (including cav-1, clathrin, adaptins) and chaperones (including Tid1, GRP58, HSP90). The possibility that disruption of cav-1/raft/STAT signaling mechanisms may represent an intiating event in the pathogenesis of a specific disease process — the development of pulmonary hypertension in the MCT-treated rat — is now receiving experimental attention. Overall, the studies reviewed above highlight an as yet largely unexplored area of cytokine/STAT signaling — the subcellular anatomy of this signaling pathway at the level of the plasma membrane raft and in the cytoplasm.

It is a privilege and a pleasure to contribute to a special issue of this journal published in

honor of Prof. Dr. Aleksander Koj. I have known Dr. Koj both as a scientist and as a friend these last fifteen years and truly value and cherish the perspective and joy that he brings to the process of doing science. It has indeed been a pleasure to be part of Dr. Aleksander Koj's scientific family. His perspective and incisive comments have served to keep us all honest and focussed on the important questions in front of us. It is the novel mixture of intellectual rigor and personal softness that makes any discussion with him so memorable. This brief review of only one aspect of recent work in the cytokine field, which is a shortened adaptation of a longer review (Sehgal & Shah, 2003), is dedicated to this man of great vision.

The XXXth Winter School of the Faculty of Biotechnology of the Jagiellonian University in Zakopane (February 28, 2003 to March 4, 2003) organized by Prof. Dr. Adam Dubin and Dr. Joanna Beretta in honor of Dr. Koj's fifty years in science was a rich and rewarding experience. The focus on giving young graduate students in the Department of Biotechnology the opportunity to discuss their observations and mix and mingle with more established scientists from around the world is to be highly commended. Well done! And, thanks for the memories.

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