Grand challenges in pharmacotherapy of inflammation for the first decades of the 21st century

Paola Patrignani*, Stefania Tacconelli and Annalisa Bruno

Department of Medicine and Center of Excellence on Aging, “G. d’Annunzio” University, School of Medicine, Chieti, Italy

*Correspondence: ppatrignani@unich.it

Inflammation is a complex set of interactions among soluble factors and cells that can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic or autoimmune injury (Nathan, 2002; Gilroy et al., 2004). Inflammation, per se, does not cause disease. But unchecked inflammation that spreads possibly due to disruption of the resolution processes can lead to chronic persistent inflammation which plays a role in the progression of many diseases, such as atherosclerosis, asthma, neurodegeneration, inflammatory bowel diseases (IBDs) and cancer. Thus, inflammation is one of the relevant areas in biomedical research. Molecular mechanisms regulating the checkpoints tuning on and off the inflammatory response have been revealed and numerous novel potential therapeutic targets have been uncovered. Humanized antibodies for anti-cytokine therapy have been shown to be effective for the treatment of rheumatoid arthritis and various inflammatory diseases (Maini and Taylor, 2000). However, there is still an important gap to be filled by developing new therapeutics which should be safer and more specific for different inflammation-related diseases.

Importantly, we need to dissect the influence of genetic background but also behavioral and psychosocial factors in different socioeconomic groups on increased susceptibility to inflammation. This knowledge will lead to predict and prevent inflammatory-related diseases and hopefully to reduce social inequalities in disease development observed in many populations (Ranjit et al., 2007).

DEVELOPMENT OF STRATEGIES TO IMPLEMENT PERSONALIZED MEDICINE OF ANTI-INFLAMMATORY DRUGS

Since the development of inflammatory-related diseases is a complex multi-step process involving many biological pathways as well as environmental factors, it will be possible to identify molecular fingerprints, of multiple genes, RNAs, proteins, metabolites, or combination of them, associated with unchecked inflammation. The ultimate goal of the genomic, proteomic, and metabolomic studies will be to clarify whether changes in the interconnecting networks of genes, proteins, and metabolites are involved in disease susceptibility and/or accelerated disease progression. These studies will lead to the discovery of novel disease biomarkers which will be very useful to improve clinical interventions and to translate into personalized strategies of treatment (FitzGerald, 2005). Thus, it will be possible to tailor commercialized medicines or novel therapeutics to the individual patient, minimizing the number of exposed individuals who fail to benefit from the treatment or experience adverse events from the treatment.

INTEGRATION OF REGENERATIVE MEDICINE INTO INFLAMMATION RESEARCH

A difficult, but possible, challenge of inflammatory research is to regenerate the impaired organs due to excessive chronic inflammation. In osteoarthritis and other degenerative diseases of the bones and joints, it will be possible to regenerate the damaged tissue by using patient’s stem cells. However, many obstacles persist, such as a suboptimal frequency and abundance of adult stem cells, or limited potency. Importantly, the environment plays an active role in promoting growth and potency of stem cells (Logan et al., 2007). The environment within the body changes hugely as you age and this represents a factor which limits stem cell maintenance. However, it is possible to modify stem cells in the lab and re-introduce them to the body. Recent findings have shown that modified adult stem cells through the introduction of cyclic AMP which activates protein kinase A (PKA), mature into bone cells when reintroduced to the body (Siddappa et al., 2008).

NOVEL INSIGHTS INTO CHRONIC INFLAMMATION AND CANCER DEVELOPMENT AND PROGRESSION

The association of chronic inflammation and cancer development and progression is becoming apparent but the mechanisms remain largely obscure. Thus, IBDs, both ulcerative colitis (UC) and Crohn’s colitis, ranks among the top three high-risk conditions for colorectal cancer (CRC), together with the hereditary syndromes of familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPPC) (Itzkowitz and Yio, 2004). Unlike the latter two conditions that have a well-defined genetic etiology, CRC risk in IBDs appears to be related more to chronic inflammation of the gastrointestinal (GI) mucosa triggered in a susceptible individual by an environmental insult such as GI infection or other environmental toxins (Itzkowitz and Yio, 2004). Recent studies have underscored a striking connection between tissue injury, repair and malignancy and systemic rheumatic diseases (Rosen and Casciola-Rosen, 2006).

A new hypothesis is that chronic inflammation may switch on the endogenous injury-repair machinery where stem cells play a critical role. It is possible that increased stem cell recruitment and mutation in the environment of chronic inflammation may translate into cancer formation in the inflamed target tissue (Houghton et al., 2004). Prostaglandin E2 (PGE2), an important mediator of inflammation which has been shown to induce somatic and embryonic cell survival (North et al., 2007), might be the link among inflammation, stem cells and cancer development.

In addition to this mechanism, PGE2 promotes cancer development by multiple other effects, ie by inhibiting apoptosis,
promoting cell growth, inducing angiogenesis and tumor invasion (Wang and Dubois, 2006). Altogether these findings suggest that inhibition of inflammatory PGE₁ may be an appropriate strategy to fight cancer (Cha and Dubois, 2007).

PGE₁ is generated from arachidonic acid (AA) after its release from membrane phospholipids by phospholipases. AA is transformed into prostaglandin (PG) H₂ through the activity of cyclooxygenase (COX) enzymes (i.e. COX-1 and COX-2) (Funk, 2001). The isomerization of PGI₂ to PGE₁ is catalyzed by three different isomerases: a cytosolic PGE synthase (cPGES) and two membrane-bound PGE₅s, mPGES-1 and mPGES-2 (Kudo and Murakami, 2005). Of these isomerases, cPGES and mPGES-2 are constitutive enzymes whereas mPGES-1 is mainly an induced isoform. PGE₁ interacts with specific receptors, termed EP₁–4, that have restricted patterns of expression and receptor-specific actions (Narumiya and FitzGerald, 2001; Kamei et al., 2003).

Inhibition of COX-2-dependent PGE₁ by nonsteroidal anti-inflammatory drugs (NSAIDs) selective for COX-2 (coxibs) has shown to have chemopreventive activity for CRC and other types of cancer (Thun et al., 2002; Cha and Dubois, 2007). However, the long-term use of coxibs is restricted by the occurrence of adverse cardiovascular events believed to be associated with the reduction of the biosynthesis of vascular COX-2-dependent prostacyclin which is an important protective pathway for the cardiovascular system (Grosser et al., 2006; García Rodríguez et al., 2008; Patrignani et al., 2008). A possible strategy to overcome the cardiovascular toxicity of COX-2 inhibition is to target the downstream enzyme in PGE₁ biosynthesis, ie mPGES-1. In fact, several evidences suggest a key role of mPGES-1 in carcinogenesis (Kamei et al., 2003; Hanaka et al., 2009).

**DEVELOPMENT OF DUAL INHIBITORS OF mPGES-1 AND 5-LO**

PGE₁ couples via high-affinity EP4 receptors and may upregulate IL-8 (Yu and Chadee, 1998). IL-8 is a potent chemokine which can attract and activate neutrophils to cause nonspecific tissue damage, important in the onset of colonic inflammation (Dey et al., 2006). Activated leukocytes alone and their interaction with other cells, mainly with platelets, via P-selectin/P-selectin glycoprotein ligand-1 (PSGL-1), are involved in enhanced generation of leukotrienes (LT) (Evangelista et al., 1999). LT₅s, ie LT₅B₅ and LT₅C₅, are important factors of inflammatory responses. They are produced from AA by 5-lipoxygenase (5-LO), functionally coupled to 5-LO-activating protein, an integral membrane protein present on the nuclear envelope that presents AA to 5-LO. Neutrophils generate the unstable intermediate LTA₅ by 5-LO; then, it is released or enzymatically hydrolyzed by LTA₅ hydrolase to produce LTB₅. When released, LTA₅ can be taken up by neighboring cells, in particular platelets, and conjugated with glutathione by LTC₅ synthase to generate LTC₅, which is further metabolized extracellularly to LTD₅ and LTE₅ (Gijón et al., 2007). Altogether these findings support an important cooperation of COX-2/mPGES-1 and 5-LO pathways in inflammatory diseases. The development of dual inhibitors of mPGES-1 and 5-LO pathways may represent a novel strategy to restrain inflammation (Koeberle et al., 2008, 2010). There is accumulating evidence that inhibition of both the LT and the PG biosynthetic pathway is superior over single interference, in terms of anti-inflammatory effectiveness and also due to a lower incidence of gastrointestinal toxicity (Celotti and Laufer, 2001; Kulkarni and Singh, 2007).

Interestingly, the pro-inflammatory 5-LO product LTD₄ is tumorgenic causing translocation of β-catenin both to the mitochondria, in which it associates with the survival protein Bcl-2, and to the nucleus in which it activates the TCF/LEF transcription machinery involved in the expression of pro-tumorigenic genes, ie COX-2, c-myc, cyclin D1, and PPARδ (Mezhybovska et al., 2006). Moreover, it has been recently shown that 5-LO gene (Alox5) may represent a novel strategy to target cancer stem cells. Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia in experimental animals (Chen et al., 2009).

**MICRORNAS AS THERAPEUTIC TARGETS**

MicroRNAs (miRNAs) are an emerging class of highly conserved, non-coding small RNAs that regulate gene expression on the post-transcriptional level by inhibiting the translation of protein from mRNA or by promoting the degradation of mRNA (Czech, 2006).

More than 800 miRNAs have been identified in mammals and increasing evidence indicates that miRNAs have distinct expression profiles and play crucial roles in various physiological and pathological processes such as cardiogenesis, haemopoietic lineage differentiation, oncogenesis, vascular disease and inflammation. miRNAs are thought to control up to 30% of all gene activity, with one type responsible for directing the expression of whole networks of genes, rather than just single genes. Several lines of evidence show that miRNAs play a key role in checking inflammation. Thus, miR146 has been proposed to target the 3′ UTRs (untranslated regions) of the TRAF6 and IRAK-1 genes, and to control Toll-like receptor and cytokine signaling through a negative feedback loop, thus reducing inflammatory cytokines production (Taganov et al., 2006). In mature human dendritic cells, miR-155 has been shown to be part of a negative feedback loop, which down-modulates inflammatory cytokine production in response to microbial stimuli (Ceppi et al., 2009). Moreover, miR-16, has been shown to contribute to AU-rich element-mediated mRNA turnover of inflammatory genes, such as TNF-α, IL-6,
and COX-2 by facilitating degradation of these transcripts (Jing et al., 2005). Thus, the identification of the actions of microRNAs adds new layers of complexity to our understanding of inflammation.

There is a great interest to develop miRNA-inhibiting drugs. Single-stranded RNA molecules have been designed to bind specifically to miR-122, which is thought to regulate up to 450 genes, around 100 of which are involved in cholesterol and lipid metabolism. This miRNA inhibitor, or antimiR, is readily taken up by liver cells and lowers cholesterol by up to 35% (Esau et al., 2006).

**NEW ERA OF PHARMACOVIGILANCE**

Effective use of medicines requires balancing the benefits and risks. No effective medicine is without risk. Most of these risks are known when a product is launched; however, some of the risks are unknown and are discovered during clinical use. In fact, pre-marketing findings regarding safety of medicines are commonly based on the experience of hundred to a maximum of a few thousand people, who have been treated in controlled randomised trials. Thus, they are too small to detect very rare events, used selected “healthy” populations and often too short to detect long-term effects. Thus, post-marketing pharmacovigilance acts an important role in patient safety. The application of pharmacoepidemiological methods together with the use of pharmacogenomics tools (Arehart et al., 2008) will open the way to a new era of pharmacovigilance (García Rodríguez et al., 2008; Massó González et al., 2010). This will allow to put information on drug hazard as quickly as possible and to make appropriate decisions on risk management.

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