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Sulforaphane regulates phenotypic and functional switching of both induced and spontaneously differentiating human monocytes

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ABSTRACT

At the site of inflammation, switching default on polarization of monocyte differentiation into classically activated macrophages (M1 type) is one of the pathogenic outcomes in several inflammatory autoimmune diseases, such as rheumatoid arthritis and osteoarthritis. In rheumatoid and osteoarthritis, a soluble collagen known as self-antigen is considered as a biomarker and acts as an important inflammatory mediator. In the present study, we investigated the effects of sulforaphane (SFN) on phenotypic changes and functional switching during *in vitro* induced and spontaneous differentiation of monocytes/macrophages, whose conditions were established with THP1 induced by PMA, and human peripheral blood monocytes, respectively. SFN at non-cytotoxic concentration (10 μM) blocked soluble collagen induced inflammatory responses specific to M1 macrophages, COX-2, iNOS, surface CD14, CD197 expressions and production of IL12p70, suggesting that signals induced by SFN eventually shifted macrophage polarization to a direction specific to M2 macrophages ($CD36_{high}$ $CD197_{extremely\ low}$). Results obtained with the induction of inflammatory conditions specific to M1 macrophages followed by SFN treatment showed that MAPKs were involved in the M1 to M2 phenotype switching. This immune-modulatory nature of SFN provides a clear indication for its ability to alleviate chronic inflammatory diseases by targeting monocytes/macrophages.

1. Introduction

Functional and phenotypic plasticity in response to changes in micro-environments is a characteristic feature of cells belonging to monocyte/macrophage lineage [1]. In general, pro-inflammatory Th1 cytokines mediate the activation of macrophages towards the classical M1 type to secrete inflammatory cytokines and chemokines mimicking the roles played by inducers of various autoimmune diseases, while Th2 cytokines alternatively shift their activation towards the anti-inflammatory M2 type to initiate Th2 humoral responses, allergic responses and wound healing following an inflammatory assault [2–4]. The M1 and M2 ratio is contemplated as an integral factor in maintaining physiological homeostasis and any uncontrolled functional and phenotype drift towards either form always resulted in disease manifestation and pathogenesis [4]. Therefore, a treatment regime that balances this adaptable behavior is considered one of the most powerful means to combat progressive immune related disease pathogenesis [5–7]. Moreover, the THP1 monocytic cell line is one of the oldest models to generate M1 or M2 macrophages *in vitro* following induction of differentiation with phorbol 12-myristate 13-acetate (PMA) [8–10].

Human peripheral blood monocytes are also capable of spontaneous differentiation *in vitro* in the presence of animal (*e.g.* FBS) or human serum without specific macrophage growth factors, such as MCSF or GMCSF [11–14]. MCSF and GMCSF induced macrophages are generally a polarized population, *i.e.*, M2 and M1 respectively [15,16]. With exposure to any exogenous polarizing factors, macrophages derived from these spontaneously differentiating monocytes display all the features of plasticity of tissue or *in vitro* induced M1 and M2 macrophages [12,13]. Therefore, this spontaneously differentiating *ex vivo* model was also used in this study.

In autoimmune diseases like collagen-induced rheumatoid arthritis (CIRA) or osteoarthritis, the peripheral monocytes at first sense, later migrate and reside at the inflamed tissues, followed by differentiation into pro-inflammatory M1 macrophages that in turn activate various collagenases influencing the degradation of articular collagens and deteriorating conditions in connective tissues further [17–22]. Under these diseased conditions, soluble degraded forms of collagens are detected either in the serum or synovial fluids [17,23,24]. The presence of these modified self-particles in the microenvironment further causes immune responses of higher magnitude that leads to persistence of disease. Therefore, in chronic arthritis, M1 subtypes and soluble collagens in synovial fluids are reported to be sensitive biomarkers [18,19].

A good therapeutic intervention for inflammatory autoimmune diseases can be developed once their therapeutic activity is well studied from an immunological context. In the recent years, several novel pharmacological interventions that can influence the polarity as well as plasticity in resident monocytes/macrophages are being studied using the CIRA models in mice. However, using such models at a prescreening stage is rather expensive and not feasible. Also, it is difficult to study

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cellular changes and mechanisms in a whole animal where immune cells are completely heterogeneous with respective participation in pathogenesis. Moreover, recent reports state that studies from such animal models may not be directly extrapolated to human conditions owing to multiple reasons [25–31]. Therefore, an *in vitro* model would be more reliable to study the mechanism of macrophage plasticity and behavior.

In vitro M1 activation has been observed when differentiating monocytes were exposed to standard doses of interferon-γ (IFNγ) and LPS [32,33], while co-treatment with interleukins IL-4 and IL-13 led to alternative activation (M2 type) of differentiating monocytes [32–34]. However, a few sporadic reports are available on how the presence of a trace amount of soluble collagens in culture media can develop collagen restricted Th1 cells that *in vitro* mimic and perpetuate collagen induced pathogenesis [35–37]. Thus, soluble collagen can be used as an *in vitro* inducer of autoimmune inflammation in a monocyte/macrophage model to screen compounds with an immune-modulatory role pre-clinically.

Hormetic phytochemical sulforaphane (SFN) or L-isothiocyanato-4-(methylsulfinyl)-butane is one of the most promising dietary, chemopreventive, cyto-protective, and distinguished member of the organo-sulfur isothiocyanate group. It is a very well-known anti-inflammatory [38–40] and anticancer agent [41–43]. Recently we proposed that SFN has an ability of cells' fate switching from survival to death and described how it remains active in its sub-apoptosis inducible concentration [44]. Previous studies documented SFN mediated induction of apoptosis in autoimmune responsive and collagen restricted T cells and fibroblasts [45,46]. Only a few other studies demonstrated significant improvements in diseased conditions in SFN treated experimental autoimmune models *via* killing self-restricted and immunogenic Th17 cells [36,45–55]. However the concept of anti-inflammatory SFN facilitating alteration in monocyte/macrophage phenotypes (expression of surface markers), plasticity (phenotype shifting without induction of cell death) and function (cytokine production) in autoimmune conditions has yet not been critically evaluated.

The objective of the current work is to study the ability of SFN to induce plasticity and phenotype switching in pro-inflammatory conditions developed *in vitro* using collagen and its implications in an autoimmune conditions. The THP1 monocytes were chosen as these cells have no prominent chromosomal abnormalities unlike other leukemic cell lines [56]. Appropriate biomarkers for M1 and M2 were used to identify and validate functional and phenotypic regulation of monocyte differentiation to macrophages upon treatment with respective inducers (collagen) either alone or with SFN [9,57–59]. Not only M1/M2 switching but also involvement of some other key immune regulators, such as p38/MEK or ERK/JNK, were also investigated because of their integral involvement in chronic inflammation as well as autoimmune arthritis associated pathogenesis [22,40,48,60].

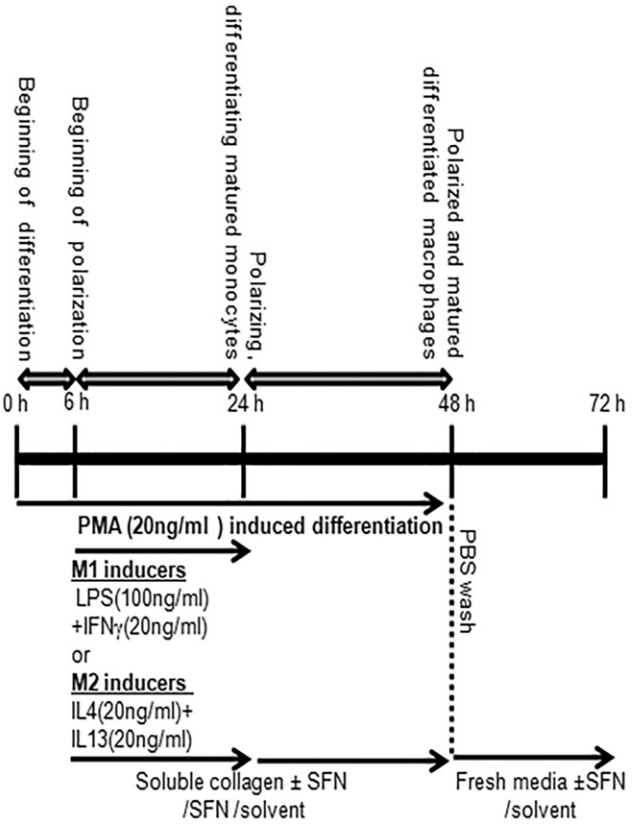
2. Material and methods

2.1. Ethics statement

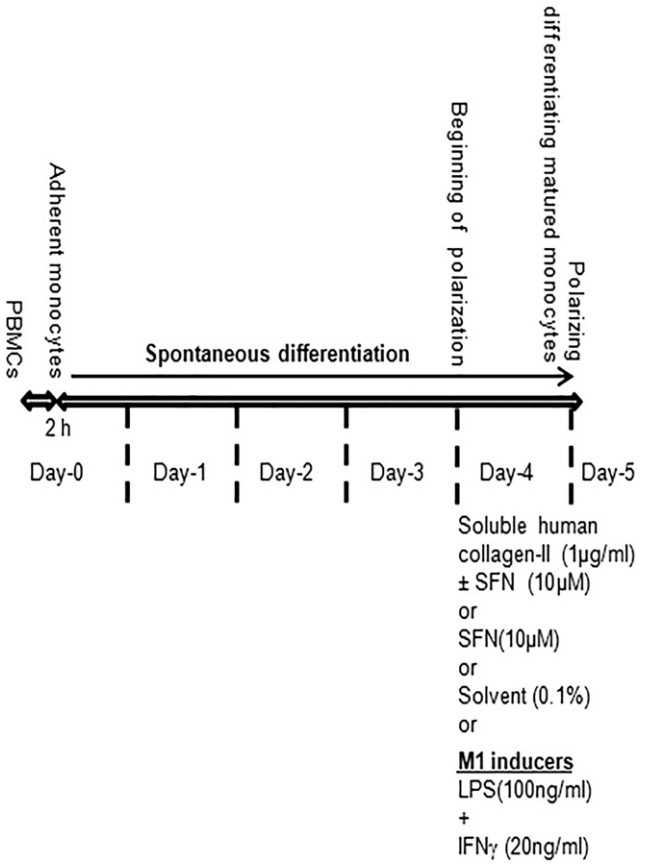
Written consents from each donor were taken by the Blood Bank Unit, Kalinga Hospital in which the blood samples were used for research purpose. Information of each human subject was protected throughout this study. For the whole studies with human PBMCs, blood was first drawn at the blood bank and PBMCs were isolated at Super Religare Laboratories (SRL), an authorized research lab stationed at Kalinga Hospital Limited by maintaining proper ethics.

2.2. Reagents and antibodies

10× PBS (#ML023), DMEM (#AL007A), RPMI (#AL162S), penicillin–streptomycin (pen–strep, Cat No: A001), and fetal bovine serum (FBS, #RM9970) were purchased from HIMEDIA. Collagen type I



Scheme 1. A schematic diagram of the work plan of the current study.



Scheme 2. A schematic diagram of the work plan for studies performed with human PBMCs.