DEVELOPMENT OF A DENDRITIC-CELL BASED ASSAY TO SCREEN MOLECULES FOR POTENTIAL ANTI-INFLAMMATORY ACTIVITY

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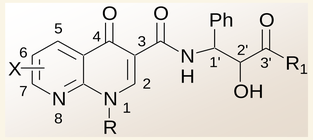
INTRODUCTION

Pro-inflammatory cytokines, such as Tumor Necrosis Factor (TNF)-α and Interleukin (IL)-1-β have been explored as potential targets in therapeutic interventions for various inflammatory disorders (1). Currently available TNF-inhibitors include monoclonal antibodies (Infliximab, D2E7) to neutralize TNF- α activity , TNF- α specific recombinant receptor construct (Etanercept), and CTLA4 fusion protein (Abatacept) to block TNF- α activity by inhibiting T cells activation (2,3).

Dendritic cells (DC), which recognize invading pathogens and translocate the processed antigens to secondary lymphoid organs for T cells activation, have been qualified as the target cells to investigate pharmacological role of various Immunomodulatory agents (4-8).

An *in vitro* septic shock assay was developed with murine bone marrow-DCs to screen new molecules for potential anti-inflammatory activity. Extent of modulation in pro-inflammatory cytokines and chemokines was taken as an indicator of anti-inflammatory activity.

Naphthyridine class of molecules has previously been reported as potential anti-inflammatory agents (9,10). We investigated the potential anti-inflammatory activity of novel derivatives of this class of molecules using *in vitro* DC based assay.



Structure of 1-8 naphthyridines derivatives

wherein X is hydrogen, halo, alkyl, alkoxy, amino or substituted amino; R is hydrogen, alkyl, aryl or heterocyclic; and R_1 is hydroxy, alkoxy, amino or substituted amino group.

OBJECTIVE

To develop an *in vitro* Dendritic-cell based assay for screening of New Chemical Entities for potential anti-inflammatory activity.

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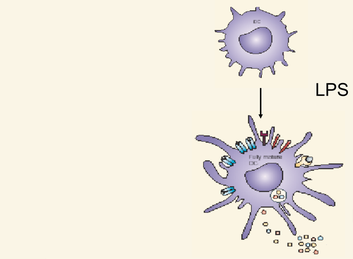
METHODOLOGY



Generation of BMDC cultures : Primary DC cultures were generated from mouse bone marrow. Bone marrow progenitor cells were cultured in presence of rmGMCSF, at 37°C, 5%CO_2. Immature DCs were preincubated with LPS, resulting in elevated levels of pro-inflammatory cytokines and chemokines.

Activity Screening of new molecules: 1-8 naphthyridine derivatives were screened from an in-house library of NCEs synthesized at DRF. Primary screening of new molecules was done at two specific concentrations 0.1μg/ml and 1 μg/ml. Molecules with potential anti-inflammatory activity were subjected to screening over a multiple dose concentration range of 0.001 μg/ml to 10 μg/ml. Supernatants were collected for analyses.

Cytokine and chemokine analyses: The panel of pro-inflammatory cytokines and chemokines analyzed using specific enzyme linked immunosorbent assay kits for modulatory activity of test compounds comprised of

- Tumor Necrosis Factor-α
- Interleukin-1-β
- Interleukin-6 (IL-6)
- Macrophage-Inflammatory Protein (MIP-1-α)
- Interferon-gamma-inducible Protein (IP-10).

Animals: Inbred 6-8 weeks old, male C57BL/6 mice were used. The animals were bred in house at the specific pathogen free Small Experimental Animals facility of Dabur Research Foundation. All animal studies were approved by the Institutional Animal Ethics Committee of Dabur Research Foundation.

RESULTS AND DISCUSSION

- BMDC based assay was developed to screen new molecules with potential anti-inflammatory activity.
- Culture conditions were optimized to obtain reproducible DC yields and minimum inter and intra assay variations.
- Assay system was validated with known anti-inflammatory agents, such as NS-398 and Indomethacin for downregulation of TNF-α , and IL-6.
- A library of 100 NCEs belonging to naphthyridine group was evaluated for anti-inflammatory activity by modulation of pro-inflammatory cytokines and chemokines.
- The extent of inhibition of elevated levels of pro-inflammatory cytokines by test molecules was indicative of their anti-inflammatory properties. The downregulation of chemokine and cytokine levels by > 25% was considered as significant.

TNF-α activity:

- Significant down regulation of TNF-α -activity was observed for compound no. #59, #62, #66, #68, and #71.
- Screening was carried out over a concentration range of 0.001-10 μg/ml for selected compounds no. #59, #62, #66, #68, and #71.
- IC_{50} values were found to be <0.001 μg/ml for these compounds.

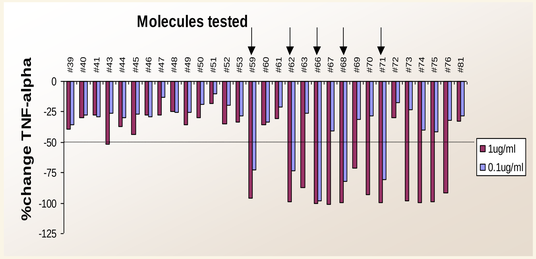


Figure-1: TNF- α downregulation

IL-1-β activity:

- Same set of molecules were analyzed for downregulation of IL-1-β activity.
- Several of the molecules demonstrated >50% IL-1β inhibitory activity.

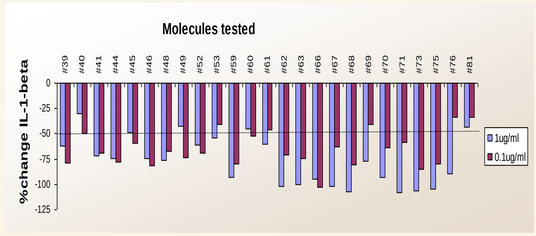


Figure-2: IL-1-β downregulation

IL-6 activity:

- Molecules exhibiting high TNF- α down modulation also demonstrated IL-6 inhibition, (IC_{50} < 0.001μg/ml).

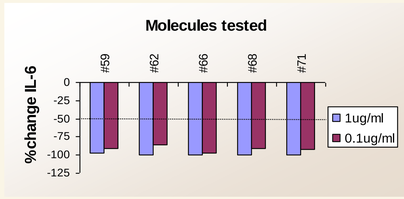


Figure-3: IL-6 downregulation

MIP-1-α activity:

- Molecules with potent TNF-α and IL-1-β activity were investigated for their effect on MIP-1-α activity, a key pro-inflammatory chemokine.
- Compound No. #59, #62, #68, #71 down regulated MIP-1-α at 0.1μg/ml and 1 μg/ml.

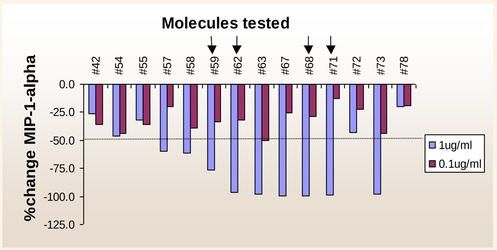


Figure-4: MIP-1-α downregulation

IP-10 activity:

- Compound No. #66 was a potent down regulator of IP-10 activity.
- Modest TNF-inhibitors #43, #47, #50, #51 showed <25% IP-10 downregulation.

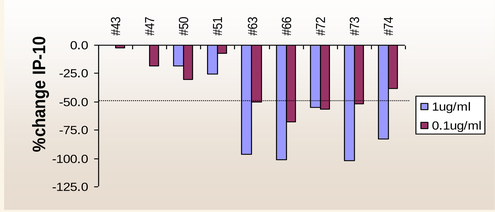


Figure-5: IP-10 downregulation

CONCLUSIONS

- This DC based *in vitro* screening can be used as a mechanism based assay for early identification of molecules with potential anti-inflammatory activity.
- Screening molecules using DC assay has resulted in early identification of potential anti-inflammatory candidates. These molecules can be further evaluated in an in-vivo model for inflammatory disorder. Hence less number of animals are required for early screening studies.
- Action of biological drugs is mediated by targeting molecular markers on cell surface, for e.g. Abatacept competes with CTLA4 on T-cells to block T cells activation. This model presents DC as an excellent cellular target for evaluation of anti-inflammatory activity and offers scope for exploring and targeting other relevant DC markers indicative of this ability.

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