Materials and Methods: Sample 1
Hultqvist M, Olofsson P, Gelderman KA, Holmberg J, Holmdahl R (2006) A New Arthritis Therapy with Oxidative Burst Inducers. PLoS Med 3(9): e348

Problem: Rheumatoid arthritis
Purpose of study: In this study we used rats as an experimental model of RA to identify compounds that increase oxidative burst capacity in vivo and investigate whether these substances thereby could have a therapeutic effect on arthritis.

Methods

Animals

Rats of strains DA and LEW.1F (Zentralinstitut für Versuchstierzucht, Hannover, Germany) were kept in a climate-controlled environment with 12 h light/dark cycles, housed in polystyrene cages containing wood shavings, and fed standard rodent chow and water ad libitum in the animal house of Medical Inflammation Research (http://www.inflam.lu.se). The rats were found to be free from common pathogens including Sendai virus, Hantaan virus, coronavirus, reovirus, cytomegalovirus, and *Mycoplasma pulmonalis*. The DA.*Ncf1*^{E3} and DA.*pia34* strains have been described [7,12]. The experiments were approved by local (Malmö/Lund, Sweden) ethical committee license M70/01 and M70/04.

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Human Promyelocytes

The human promyelocyte line HL-60 (CCl-240; ATCC, Manassas, Virginia, United States) was cultured in D-MEM (Gibco, Paisley, UK) complemented with Hepes, 5% fetal calf serum, and penicillin-streptomycin at standard cell culture concentrations. The cells were differentiated to granulocytes by culture in the presence of 1.25% DMSO (Sigma-Aldrich, St. Louis, Missouri, United States) for 6 d [13]. Before they were assayed the cells were washed and resuspended in D-PBS (Gibco) to a concentration of 10^7 cells/ml.

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Oxidative Burst Assay of Granulocytes In Vitro

Saturated alkane molecules (C8-C17) (Larodan Fine Chemicals AB, Malmö, Sweden), pristane (2,6,10,14-tetramethylpentadecane), and phytol (3,7,11,15-Tetramethyl-2-hexadecen-1-ol) (all from Sigma-Aldrich) were tested for oxidative burst-inducing capacity according to a previously described method [14]. Oils were solubilized by dilution at 1%–5% concentration in 10% β-cyclodextrin (Sigma-Aldrich) in PBS. β-cyclodextrin by itself had no stimulating effect on ROS production. Briefly, 5 μl of resuspended oils were added to 96-well plates containing 5 × 10^5 cells/well in a total volume of 200 μl of PBS containing isoluminol and horseradish peroxidase (final isoluminol concentration 100 mg/ml; Sigma-Aldrich) and horseradish peroxidase type II (5 units/ml; Sigma-Aldrich). Samples were gently mixed and data collection was initiated immediately. Extracellular ROS production was followed at 37 °C as luminescence signal (FluoStar Optima,

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BMG Labtechnologies, Offenburg, Germany) and presented as maximal relative signal during a measurement period of 30 min.

Induction and Evaluation of Arthritis

Disease was induced in all rats at the age of 6–12 wk. Rats were sex- and age-matched within all experiments.

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Treatment of Arthritis

Unless stated otherwise, preventive treatment of arthritis was performed by SC injections of 200 μl of phytol, C11, or C16 5 d before induction of arthritis. Control rats were left untreated.

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Determination of Oxidative Burst Activity Ex Vivo

The level of intracellular oxidative burst ex vivo was measured by preparing single-cell suspensions from blood, spleen, draining lymph nodes (LNs), or bone marrow (BM). Red blood cells were lysed with ammonium chloride (pH 7.4) at a concentration of 0.84%.

Oxidative burst in granulocytes and T cells was determined by incubation of cells for 30 min at 4 °C with biotin-labelled antibody HIS-48 (anti-granulocytes) or PerCP-labelled R73 antibody (anti-T cell receptor) (BD Biosciences Pharmingen, San Jose, California, United States). After they were washed with PBS, cells were incubated with allophycocyanin-conjugated streptavidin (BD Pharmingen) for 20 min at 4 °C. To determine the level of NADPH activity we used a modified version of the oxidative burst activity flow cytometry assay previously described [17]. Briefly, cells were resuspended in Dulbecco's complete medium without FCS after staining, and incubated for 10 min at 37 °C with 3 μM dihydrorhodamine-123 (Molecular Probes, Leiden, The Netherlands), which, after oxidization by hydrogen peroxide (H_2O_2), peroxinitrite ($ONOO^-$) and hydroxyl radicals (OH•) to rhodamine-123, emits a bright fluorescent signal upon excitation by blue light. Cells were then stimulated with 200 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) for 20 min at 37 °C. After a wash with PBS they were acquired on a FACSort (BD Biosciences), gated on cell-type. R-123 fluorescence intensity was measured on FL-1 and results expressed in relative fluorescence units.

Lipid Peroxidation

Ex Vivo Analysis of Lymphocyte Populations

Total IgG and IgM Levels

Ex Vivo Cell Death Assay

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Comment: COOK BOOK! Succinct writing, packed with information.

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Comment: Methods section can be very long. Using informative subheadings is very helpful. **Note that headings don't describe a result—only a method.** Organize methods in the order in which the experiments are described in the Results section. Note

Antibody Response against CII

Delayed-Type Hypersensitivity in response to CII

Determination of Serum Levels of Cartilage Oligomeric Matrix Protein

Reversion of the Phytol Effect with Histamine Dihydrochloride

Histological Analysis

Adoptive Transfer of Arthritogenic Spleen Cells

Statistics: Quantitative data are expressed as mean ± standard error of the mean, and significance analysis was performed using Mann-Whitney test. All results were compared to those from the control group unless otherwise indicated.

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