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Selective lymphocyte killing by reactive oxygen species (ROS)

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Introduction

It has been suggested that Reactive Oxygen Species (ROS) (O_2^-, H_2O_2, $OH^\cdot$), contribute to the tissue injury associated with inflammatory arthritis. Synovial constituents exposed *in vitro* to ROS generating systems such as the xanthine-xanthine oxidase system, U.V. irradiation or activated polymorphs, undergo structural alterations similar to those detectable *in vivo* [1, 2]. Besides a direct role in tissue injury, ROS released by activated phagocytes may affect the function of lymphocytes entering sites of inflammation. It might be expected that non-toxic levels of ROS would inhibit lymphocyte function, while higher levels might be directly cytotoxic. Furthermore, a differential susceptibility may exist between lymphocyte subpopulations.
U.V. irradiation of culture medium was used to create a high and sustained ROS environment in order to study the latter possibility.

Methods

Peripheral blood mononuclear cells (PBMC) were isolated from the fresh venous blood of healthy human volunteers and separated on Ficol-Paque gradients.
RPMI 1640 culture medium was irradiated at 15 minute intervals up to 1 hour, prior to addition of 10% foetal calf serum (FCS) and PBMC (2×10^6/ml). Cultures were incubated at 37 °C for 24 hours. Viability was then assessed by trypan blue dye exclusion.
To identify the oxidant species mediating PBMC killing, cell cultures were supplemented with antioxidants at the following final concentrations: Superoxide Dismutase (SOD) – 100 μg/ml; Catalase – 500 μg/ml; Mannitol – 50 mM; Thiourea – 50 mM, Desferrioxamine – 0.5 mM.
Levels of O_2^- and H_2O_2 generated in irradiated medium were measured according to [3]. Briefly, O_2^- levels were assayed by the degree to which SOD inhibits the oxidation of cytochrome c as measured by absorbance at 550 nm. H_2O_2 measurements were based on the Horse Radish Peroxidase (HRPO) dependent oxidation of Phenol Red solution. Values were calculated from O.D. readings at 610 nm against a standard reference graph.
H_2O_2 was added to PBMC in a concentration range dependent on the values obtained in the phenol red assay. Viability at 24 hours was assayed as above.
Selective PBMC killing by ROS was examined using ethidium bromide and FITC-staining. This allowed phenotypic characterisation of viable cells at two levels of killing: a minimal level (10–30%), where PBMC were incubated in medium irradiated for 5 minutes; approximately 50% killing, where PBMC were incubated in medium irradiated for 15 minutes. All fluorescence was done in solution. B cells were identified by direct FITC-staining using sheep α-human μ-chains and T cells by indirect immunofluorescence [4].

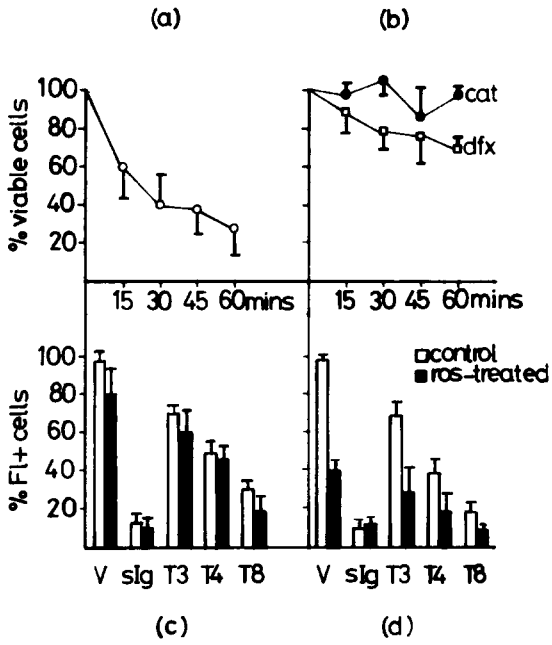

Figure 1
(a) Cytotoxic effects of U.V. irradiated RPMI 1640 for PBMC. (b) Cytotoxic effects of U.V. irradiated RPMI 1640 for PBMC in the presence of antioxidants: Catalase (cat); Desferrioxamine (dfx). (c) Phenotypic profile of viable PBMC after exposure to RPMI 1640 irradiated for 5 minutes. (d) Phenotypic profile of viable PBMC after exposure to RPMI 1640 irradiated for 15 minutes. V = viable cells.

Results and discussion

A dose-dependent relationship existed between PBMC killing and the length of time that culture medium was irradiated. Of the antioxidants used, only Catalase, an enzyme which catalyses the breakdown of H_2O_2 to H_2O and O_2 gave significant protection ($p < 0.001$). However, desferrioxamine by chelating ferric iron reduced the availability of catalytic ferrous iron required for $OH^{\cdot}$ radical generation (Fenton reaction):

$$(Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}).$$

The significant protection by desferrioxamine ($p < 0.001$) suggested that H_2O_2 was toxic via $OH^{\cdot}$ radicals. H_2O_2 was shown to be the predominent ROS generated by U.V. irradiation, while O_2^{-} remained at a low steady state concentration. H_2O_2 added exogeneously, at concentrations comparable to that generated by U.V. irradiation, produced a similar pattern of PBMC killing.

Fluorescent staining of viable cells indicated that T lymphocytes were the cells most susceptible to oxidant induced killing and that T8+ cells were particularly susceptible at low levels of ROS. With increasing levels of ROS, a significant loss of T4+ cells occurred and a small viable T8+ population remained.

Non-cytotoxic levels of H_2O_2 have previously been shown to selectively abrogate T cell function *in vitro* [5]. This has led to the proposal that ROS may be involved in modulating the immunological responses associated with inflammatory disorders such as Rheumatoid Arthritis. This study suggests that, in an environment where oxidant levels exceed those required to depress lymphocyte function, as may be the case in the rheumatoid synovium, selective depletion of lymphocyte subpopulations by ROS may also play an important role in determining the overall immunological status of the synovium.

References

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