Xenon has no effect on cytokine balance and adhesion molecule expression within an isolated cardiopulmonary bypass system†‡

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Background. Although almost inert chemically, xenon is not unreactive biologically. It interacts with receptors involved in the expression of cytokines and adhesion molecules. The effect of xenon on the immune function in whole blood has not been studied.

Methods. We examined the effects of 70% xenon in oxygen on cytokine balance and expression of adhesion molecules in an isolated cardiopulmonary bypass (CPB) system, which simulates an evolving inflammatory response. Whole blood from 10 healthy male volunteers was circulated in a CPB system supplied with either 70% xenon in oxygen, or oxygen-enriched air – FO2=0.3 (control). We took samples of blood after 30, 60 and 90 min of simulated CPB. We measured interleukin (IL)-1β, tumour necrosis factor (TNF)α, IL-8, IL-10, IL-1ra and TNF-sr-2 levels, and the expression of HLA-DR and the adhesion molecules L-selectin, CD18 and CD11b on monocytes, granulocytes and lymphocytes.

Results. IL-8 concentrations were increased significantly, TNF-sr-2 concentrations decreased significantly and IL-10 levels decreased during bypass. There were no significant differences between the groups for any measured variable.

Conclusion. In an isolated CPB system, xenon and oxygen-enriched air had similar effects on cytokine production and expression of adhesion molecules.

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Xenon is an inert gas with potent anaesthetic and analgesic properties which could replace nitrous oxide. It acts on the N-methyl-D-aspartate (NMDA) receptor1 and interferes with intracellular calcium homeostasis.2 3 Because both these mechanisms are involved in cytokine and adhesion molecule expression, it is possible that xenon could modify the normal human immune response to inflammation.

Extracorporeal cardiopulmonary bypass (CPB) simulates an intense, progressive inflammatory response which includes complement activation, increased adhesion molecule expression and an increase in the plasma concentrations of pro-inflammatory cytokines. We have used this isolated CPB model to study the effects of various anaesthetic medications on the human immune system.4

Because the effects of xenon on immune function are not known, we used the same CPB system to compare the effects of 70% xenon in 30% oxygen with oxygen-enriched air (FO2=0.3) on the production of L-selectin, HLA-DR, CD18, CD11b, interleukin (IL)-1β, tumour necrosis factor (TNF)α, IL-8, IL-10, IL-1ra and TNF-sr-2 during 90 min of simulated CPB.

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Methods
After obtaining local research ethics committee approval and written informed consent, 10 healthy male volunteers, who were not receiving any medication and had refrained from alcohol and caffeine for 24 h, each donated two 250-ml samples of blood into standard blood donation bags containing SAG-M anticoagulant. Heparin (1000 IU) was added to each bag. The donated blood was immediately circulated in one of two identical CPB circuits each primed with 270 ml Hartmann’s solution. A trained perfusionist commenced simulated bypass at 37°C. Flow through the bypass apparatus was maintained at 2.5 litre min⁻¹. The membrane oxygenator of each bypass apparatus was then supplied with either 70% xenon in 30% oxygen, or oxygen-enriched air (FrO₂=0.3). Each membrane oxygenator was also supplied with carbon dioxide using a calibrated flowmeter, as required, to maintain the partial pressure of carbon dioxide within the blood between 4 and 6 kPa. The fractions of gas delivered to each membrane oxygenator were monitored using a Capnomac Ultima (Datex Engstrom, Helsinki, Finland) and the xenon concentration was monitored using a thermal conductivity meter calibrated for the measurement of xenon in oxygen (Bedfont Scientific, Rochester, UK).

A 10-ml sample of blood was taken from each bag after donation (time 0) and from each membrane oxygenator at 30, 60 and 90 min. Samples were processed immediately. Four 0.1-ml aliquots of whole blood were stained using monoclonal antibodies for CD18, CD11b (Flow Cytometry Primary Antibodies and Conjugates, Dako Ltd, Denmark), L-selectin, HLA-DR and mouse IgG [control] (Becton, Dickinson and Company, NJ, USA). The whole blood was then lysed to remove the red cells, thus permitting the expression of surface markers on white blood cells to be determined using flow cytometry. Quantification of the adhesion molecules was carried out immediately after acquisition and processing of the samples, by a laboratory technician skilled in this field. Flow cytometric analyses were performed on an EPICS ELITE instrument (Coulter Electronics Ltd, Luton, UK). This instrument was calibrated before each series of analyses by a technician trained in this method, using Immuno-Check alignment fluorospheres (Coulter Corporation, Hialah, FL, USA). Two thousand cells were analysed and displayed as dot histograms for each assay. Following subtraction of the background staining from the control samples, the percent positivity and mean channel fluorescence (MCF) intensity of the respective cell populations was determined.

At the same time, the second 250-ml sample of blood was centrifuged to separate the plasma, which was aliquoted and stored at −70°C for future analysis of the pro-inflammatory cytokines IL-1β, TNFα, IL-8 and the anti-inflammatory cytokines IL-10, IL-1ra and TNF-sr-2. A commercially available enzyme-linked immunosorbent assay (ELISA) (Quantikine™, R&D Systems, Abingdon, UK) was used for all cytokine assays.

Statistical analysis
The normality of the data was inspected and subsequent statistical analyses were performed using GraphPad Prism™

![Graph](https://academic.oup.com/bja/article-abstract/89/4/546/308397/0083897)

Fig 1 The mean channel fluorescence (MCF), expressed as linear units, of monocyte HLA-Dr, CD18, CD11b and L-selectin in donor blood at baseline (time 0) and after 30, 60 and 90 min of isolated CPB with xenon in oxygen, and with oxygen-enriched air (control). The error bars represent 1 SD.
Fig 2 The mean channel fluorescence (MCF), expressed as linear units, of granulocyte HLA-Dr, CD18, CD11b and L-selectin in donor blood at baseline (time 0) and after 30, 60 and 90 min of isolated CPB with xenon in oxygen, and with oxygen-enriched air (control). The error bars represent 1 SD.

Fig 3 The mean channel fluorescence (MCF), expressed as linear units, of lymphocyte HLA-Dr, CD18, CD11b and L-selectin in donor blood at baseline (time 0) and after 30, 60 and 90 min of isolated CPB with xenon in oxygen, and with oxygen-enriched air (control). The error bars represent 1 SD. Significant differences from time 0: **P<0.01; ***P<0.001.

version 3.0. Adhesion molecule expression and cytokine data were normally distributed and were analysed using repeated measures ANOVA and Bonferroni’s post-test correction. The number of bypass experiments done was based on a previous similar study in which 10 bypass experiments were needed to show a difference in IL-8 levels
of 16 pg ml\(^{-1}\) after 90 min (power of 90%, \(\alpha=0.05\)). \(P<0.05\) was considered statistically significant.

**Results**

Leucocyte adhesion molecule expression (HLA-DR, L-selectin, CD18 and CD11b), expressed as the MCF intensity in linear units, before and during simulated CPB is shown for monocytes, granulocytes and lymphocytes in Figures 1–3, respectively. There were significant reductions in lymphocyte CD18 and CD11b in both groups during simulated bypass. The expression of HLA-DR in granulocytes increased from baseline in both groups by 90 min of bypass, significantly so (\(P<0.05\)) in the xenon group, but there were no between-group differences at any time point.

The effect of xenon on the pro-inflammatory cytokines IL-1\(\beta\), TNF\(\alpha\), IL-8 and the anti-inflammatory cytokines IL-10, IL-1ra and TNF-sr-2 is shown in Figures 4 and 5, respectively. Compared with baseline, in both groups, at 90 min, IL-8 concentrations were significantly elevated (two samples were excluded from statistical analysis because of a technical error), TNF-sr-2 concentrations were significantly reduced after the start of simulated bypass and IL-10 levels decreased in both groups during bypass. IL-1ra was
unchanged in both groups during bypass compared with baseline. IL-1β concentrations also showed no within- or between-group differences. TNFα levels decreased after the start of bypass to below the limit of the assay. There were no significant differences between the groups for any variable. Qualitative observation during flow cytometry showed a transient reduction in monocytes only, which recovered by 90 min. This was observed in four of the 10 xenon regimens and in five of the 10 control regimens. In all others the cell numbers did not change.

Discussion
During CPB, rapid changes in plasma cytokine concentrations and circulating lymphocyte populations occur. These changes may be affected by pharmacological interventions and anaesthesia.4-6 The exact mechanisms and clinical significance of the immunomodulatory changes during CPB are not fully understood. During in vivo cardiac surgery, blood sampling alone may not be a satisfactory way to evaluate the immunomodulatory effect of any drug. It is known that bone marrow changes during CPB are reciprocal to the blood changes, suggesting that the T cells have gone out of the blood system to the marrow and possibly the lymphatic system.7 Thus, blood changes may not indicate immunosuppression but rather an appropriate immunological response. For example, many cytokines that increase with CPB can up-regulate expression of high endothelial venule adhesion molecule, a mechanism of translocation of leucocytes from the vascular to the lymphatic compartments.8,9 Leucocytes affected by the drug under investigation could migrate out of the circulation and escape detection by blood sampling.

One method of overcoming this difficulty is with the isolated CPB model, which has several advantages over in vivo blood sampling. Firstly, using the CPB model, changes to leucocyte sub-populations or surface markers reflect changes within the total leucocyte population in the donor blood sample, except for leucocytes which have been lysed during the CPB process or have adhered to the plastic surface. In other words, in the isolated CPB model, functional leucocytes have no hiding place from the investigator.

Secondly, this system simulates several elements of an evolving inflammatory response (complement fixation and IL-8 production as well as up-regulation of leucocyte adhesion molecule). Some extrapolation between these in vitro results and clinical effect may be made. In the isolated bypass system, methylprednisolone, a known immunosuppressant, causes an approximately fourfold decrease in plasma IL-8 levels.5 In vivo, a sixfold reduction in IL-8 was found at a similar time point.10 This difference in magnitude of response between the in vitro and in vivo situations may be partly explained by the lack of anti-inflammatory response generated by this isolated bypass system.

We did not find significant immunomodulatory effects of xenon in the isolated CPB system. There were no significant between-group differences in HLA-DR or the adhesion molecules, and there were no between-group differences for any cytokine values. The fact that IL-8 concentrations increased significantly from baseline in both groups by 90 min of isolated CPB, and IL-10 and TNF-r2 levels decreased in both groups after 15 min of simulated bypass supports our previous descriptions of the inflammatory response in this model and shows an inflammatory challenge. The adhesion molecule 1-selectin is decreased in all cell lines in the xenon regimen compared with the control regimen. This did not achieve statistical significance at any point but is a trend that merits further investigation.

Our results suggest that, as far as immunomodulation is concerned, xenon appears as safe as oxygen-enriched air.

References