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Anti-CD8 treatment reduces the severity of inflammatory arthritis, but not vasculitis, in mercuric chloride-induced autoimmunity

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SUMMARY

Mercuric chloride ($HgCl_2$) induces a T cell-dependent autoimmune syndrome in Brown-Norway (BN) rats characterized by a humoral response, tissue injury with an accumulation of $CD8^+$ and $CD4^+$ T cells, and an increase in tissue IL-4 mRNA and serum IgE suggesting Th2 cell activation. In other models of autoimmune disease, $CD8^+$ cells act in both anti- and pro-inflammatory capacities, suggesting that functionally distinct $CD8^+$ populations exist *in vivo*. The effect of treatment with OX8, a depleting anti-CD8 MoAb, on the initiation of $HgCl_2$-induced autoimmunity was assessed in two experiments in a total of 20 BN rats, and compared with 20 animals treated with a control MoAb or PBS. OX8 significantly depleted peripheral blood $CD8^+$ lymphocytes, had no effect on $HgCl_2$-induced anti-collagen or myeloperoxidase antibodies, nor on the incidence or severity of caecal vasculitis. The severity of $HgCl_2$-induced arthritis was significantly reduced in OX8-treated animals; median peak score reduced from 7·5 to 3·0 (experiment 1) and from 7·0 to 4 (experiment 2) ($P = 0{\cdot}009$, Mann–Whitney *U*-test). OX8 treatment also exacerbated the early rise in $HgCl_2$-induced IgE and induced a significant rise in plasma interferon-gamma (IFN-γ), suggesting that $CD8^+$ cells may have a regulatory influence on Th cell populations. These data provide direct evidence that $CD8^+$ cells may act in a proinflammatory capacity in both this model of autoimmunity and the pathogenesis of inflammatory arthritis.

Keywords CD8 arthritis vasculitis Brown-Norway rat mercuric chloride

INTRODUCTION

Mercuric chloride ($HgCl_2$) induces a syndrome of autoimmunity in Brown-Norway (BN) rats characterized by a variety of IgG autoantibodies, very high concentrations of serum IgE, proteinuria, leucocytoclastic vasculitis which predominantly affects the caecum, and an inflammatory polyarthropathy [1–4]. The kinetics of both the serologic and tissue changes follow a distinctive pattern that may be divided into three phases: (i) initiation, ≈10 days after the start of $HgCl_2$ treatment; (ii) autoregulation, resulting in complete resolution 1–2 weeks later; and (iii) resistance to subsequent rechallenge with $HgCl_2$. The initiation of the humoral response, proteinuria, vasculitis and arthritis have been shown to be T cell-dependent [4–7] and the association with an increase in IL-4 mRNA [8,9] and high concentrations of total IgE [1] suggest Th2 cell activation. However, the individual role of T cell subsets, such as $CD4^+$ and $CD8^+$ cells, in the pathogenesis of $HgCl_2$-induced vasculitis and arthritis ($HgCl_2$ A) is unknown.

In this model treatment with OX8, a depleting anti-CD8 MoAb, inhibits the resistance of the humoral response to rechallenge with $HgCl_2$ [10], suggesting that $CD8^+$ cells have an immunosuppressive role in the resistant phase. Other reports of OX8-treated BN rats have not demonstrated a significant difference in the initiation or autoregulation of the primary humoral response induced by $HgCl_2$ [10,11], even though an increase in $CD8^+$ cells occurs in peripheral blood, joints and other tissues at the time of peak disease [4,12].

Evidence from other models of autoimmunity suggests that $CD8^+$ cells perform both effector and regulatory functions in the pathogenesis of autoimmmune disease [13–19]. In view of this, our aim was to determine whether $OX8^+$ cells played a role in the induction or regulation of $HgCl_2$-induced arthritis and vasculitis, plasma anti-myeloperoxidase (MPO) and anti-collagen (type I) antibodies, total IgE and interferon-gamma (IFN-γ) concentrations in BN rats.

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MATERIALS AND METHODS

Animals, treatment protocols and experimental design

BN rats (150–400 g) were obtained from Harlan/Olac (Bicester, UK), given food and water *ad libitum* and used in age- and sex-matched groups. All experimental procedures and clinical evaluations were performed under halothane anaesthesia.

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The murine OX8 (IgG1 anti-rat CD8) MoAb producing cell line was obtained from the European Collection of Animal Cell Cultures. IgG1 antibody was purified from tissue culture supernatant using protein A affinity chromatography. OX8 recognizes and depletes rat $CD8^+$ lymphocytes and in high concentration *in vivo* has also been found to deplete rat natural killer (NK) cells and inhibit their function [20]. The FITC-labelled anti-rat NKR-P1 antibody, which recognizes rat NK cells and polymorphs, was purchased from Endogen (Cambridge, MA).

Two experiments were performed. In experiment 1, 10 BN rats received OX8 (100 μg in 0·5 ml PBS, i.v.), five received the same amount of MOPC-21 (murine isotype control MoAb; Sigma, Poole, UK) and five received PBS (0·5 ml, i.v.) on days -3, 0, 4, 9 and 14. The same group sizes and MoAb doses were used in experiment 2, but injections were performed on days -3, 0, 4, 7, 11 and 14. All animals received $HgCl_2$ (BDH Chemicals, Poole, UK) as a 0·1% solution in distilled water on 5 alternate days (1 mg/kg per day, s.c.) starting on day 0. Animals were bled from the tail artery into pre-heparinized syringes (1000 U/ml) on each day MoAbs or PBS were administered and on day 16 (exp. 1) or 17 (exp. 2) for peripheral blood $OX8^+$ lymphocyte analysis. Plasma samples were stored at -20°C for antibody and IFN-γ analysis by ELISA.

Clinical evaluation

Arthritis and caecal vasculitis were scored macroscopically by an experienced observer unaware of treatment groups. Arthritis was scored during peak disease, on days 12–16 in experiment 1 and on days 12–18 in experiment 2. On each day a score was recorded from 0 to 3 for each foot [4]. An animal was defined as having developed arthritis if any joint scored 2 or more on any day. The peak arthritis score was the highest daily score recorded on any day for each animal. The cumulative arthritis score was the sum of the daily arthritis scores for each animal.

The serosal and mucosal appearances of the caecum were scored for vasculitis in experiment 1 on day 16 and in experiment 2 on day 18. This scoring system previously has been found to correlate well with a histological vasculitis score [21].

Peripheral blood lymphocyte subset and NK cell analysis

Heparinized peripheral blood samples (200 μl) were incubated at 37°C with Tris-buffered ammonium chloride (pH 7·2, Boyle's solution) to lyse the erythrocytes. The remaining cells were suspended in PBS, 1% bovine serum albumin (BSA), 0·1% sodium azide (PBAz) and incubated in equal aliquots in polystyrene tubes (Falcon 2052) with OX8 or MOPC-21 (final concentration 0·2 μg/ml) for 15 min at room temperature. The cells were washed in PBAz and incubated with an anti-mouse, rat-adsorbed, IgG FITC-conjugated antibody (F2266; Sigma), diluted 1 : 2000 in PBAz (15 min, room temperature), washed again and resuspended in PBAz. The proportion of $OX8^+$ cells was calculated using a fluorescence-activated cell sorter (FACS; Becton Dickinson, San Jose, CA), gating on the lymphocyte population and subtracting the $MOPC\text{-}21^+$ population. Cells were also incubated with the FITC-conjugated anti-rat NKR-P1 antibody (5 μl per 1×10^6 cells in PBAz) and the characteristics of the $NKR\text{-}P1^+$ population in relation to the $OX8^+$ population were ascertained using FACS analysis.

Anti-collagen (type I), MPO and total IgE ELISAs

Anti-collagen (type I) antibodies and total IgE were measured by ELISA as previously described [4,22]. The ELISA protocol that was used for type I collagen was also used to measure anti-MPO antibodies, except that plates were coated with human MPO (a gift from Dr C. M. Lockwood, Addenbrooke's Hospital, Cambridge, UK) at 4 μg/ml in carbonate coating buffer. Test plasma results were expressed as the mean absorbance from antigen-coated wells in duplicate minus the absorbancy from the antigen-free well as a proportion of the same calculation for positive control wells on the same plate.

Rat IFN-γ ELISA

Plates were coated with 50 μl monoclonal anti-rat IFN-γ (DB1, a gift from Dr P. van der Meide, TNO, Rijswijk, The Netherlands) diluted to 5 μg/ml in carbonate coating buffer, or with coating buffer alone, incubated at 4°C overnight and then washed three times in PBS. Unoccupied binding sites were blocked with 5% skimmed milk ('Marvel', Stafford, UK) for 2 h at 4°C and plates were then washed three times in PBS, 0·1% Tween-20 (PBS–T). A standard concentration range (0·01–1000 ng/ml) of rat IFN-γ (a gift from Dr P. van der Meide) and test rat plasma samples diluted 1 : 10 in PBS, 1% BSA, 0·1% Tween-20 (PBS–B–T), were added (50 μl) in duplicate to the anti-IFN-γ-coated wells and singly to anti-IFN-γ-free wells, incubated at 37°C for 1 h and then washed with PBS–T. Plates were then incubated with polyclonal rabbit anti-rat IFN-γ (a gift from Dr J. Tite, Wellcome, Beckenham, UK) diluted 1 : 1000 in PBS–B–T at 37°C for 1 h, washed as before and finally incubated at 37°C with alkaline phosphatase-conjugated goat anti-rabbit IgG MoAb (A9919; Sigma) diluted 1 : 1000 in PBS–B–T. Plates were washed again and 50 μl of *p*-nitrophenyl phosphate (Sigma) diluted to 1 mg/ml in substrate buffer were added to each well. The subsequent optical density (OD) in each well was read at 405 nm on a Dynatech MRX plate reader.

The ODs for each sample were corrected by subtracting the anti-IFN-γ-free well OD from the mean OD of the anti-IFN-γ-coated wells. A standard curve from the corrected mean ODs of the known concentrations of IFN-γ on each plate was constructed and used to calculate the corresponding IFN-γ concentration of the test samples.

Statistical analysis

Data were analysed using the computer program Instat 2 version 2.04a (GraphPad Software) using two-tailed probability criteria.

RESULTS

Peripheral blood lymphocyte subset and NK cell analysis

The FACS profile of the OX8-labelled whole blood leucocytes revealed three populations with absent, mid-intensity and high--intensity fluorescence. The mid-intensity population was identified as NK cells and the high-intensity population as lymphocytes. The NK cell population was numerically unaffected by *in vivo* OX8 treatment at any of the time points in these experiments (data not shown). In animals treated with $HgCl_2$ + MOPC-21 or PBS the proportion of $OX8^+$ peripheral blood lymphocytes (PBL) remained at $\approx$5% throughout both experiments until day 16 when it rose to $\approx$7·5%. In animals that were treated with OX8 *in vivo* the proportion of $OX8^+$ PBL fell to $\approx$0·3% on day 0 and remained suppressed until day 14 when it started to rise (Fig. 1), but remained significantly lower than that in the MOPC-21- and PBS-treated animals at all time points that were analysed ($P < 0{\cdot}01$, Mann–Whitney *U*-test).

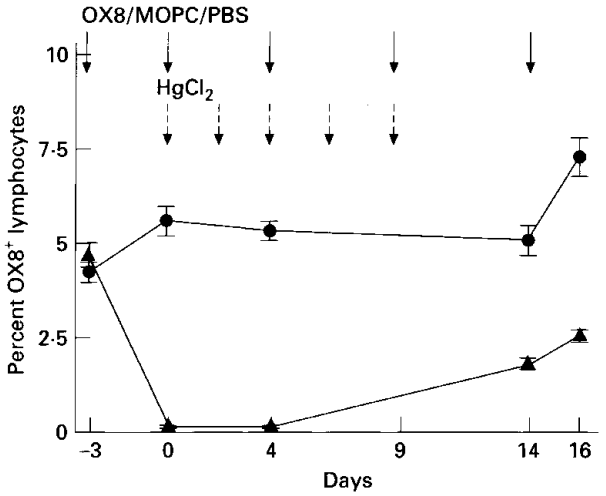

Fig. 1. Percent $OX8^+$ peripheral blood lymphocytes (PBL) in BN rats treated with either PBS/MOPC-21 ($n = 10$, ●) or OX8 ($n = 10$, ▲) in experiment 1. Solid arrows indicate the days of MoAb or PBS treatment, dotted arrows indicate the days of mercuric chloride ($HgCl_2$) treatment. Bars represent the mean scores, error bars the s.e.m.

Arthritis and caecal vasculitis

The incidence of arthritis was not significantly different between animals treated with $HgCl_2 + OX8$, MOPC or PBS (Table 1) and there was also no difference in severity of arthritis between animals treated with $HgCl_2 + PBS$ or MOPC-21 in either experiment. The severity of arthritis, as assessed by both peak and cumulative arthritis scores, was significantly lower in animals treated with $HgCl_2 + OX8$ compared with those treated with $HgCl_2 + PBS/MOPC$ in both experiments (Table 1). This is illustrated in Fig. 2, which shows the consistently lower arthritis scores in the $HgCl_2 + OX8$ group (experiment 1).

There was no significant difference in the incidence or severity of caecal vasculitis between any of the treatment groups in either experiment; total serosal + mucosal vasculitis scores from experiment 1 are illustrated in Fig. 3.

Anti-MPO and collagen (type I) antibodies

The titre of both anti-MPO and anti-collagen (type I) antibodies reached a peak at day 14 in animals treated with $HgCl_2 + MOPC$ or PBS, and neither this nor the subsequent regulation was significantly affected by treatment with OX8 (data not shown).

Plasma total IgE concentration

There was a large rise in total plasma IgE concentration in animals treated with $HgCl_2 + PBS$, $HgCl_2 + MOPC\text{-}21$ and $HgCl_2 + OX8$ in both experiments, and there was no significant difference in concentration of IgE at any time point between animals treated with $HgCl_2 + PBS$ and $HgCl_2 + MOPC\text{-}21$. The concentration of IgE in both experiments was significantly higher in animals treated with $HgCl_2 + OX8$ on days 4 and 7 (experiment 1, day 4, $P < 0{\cdot}0001$; experiment 2, days 4 and 7, $P < 0{\cdot}0022$; Mann–Whitney *U*-test) compared with those treated with $HgCl_2 \pm$ MOPC-21, but neither the peak IgE concentration at day 13–14 nor the subsequent autoregulation were significantly affected by OX8 treatment (Fig. 4, experiment 2).

Plasma IFN-γ concentration

The linear range (on a semi-log plot) of the IFN-γ standard curve was from 1 to 1000 ng/ml (not shown). There was no detectable rise (>1 ng/ml) in plasma IFN-γ at any time in animals treated with $HgCl_2 \pm$ MOPC-21 in either experiment. In contrast there was a reproducible rise in plasma IFN-γ in all animals treated with $HgCl_2 + OX8$ in both experiments (Mann–Whitney *U*-test comparison of sum of daily IFN-γ concentrations for each individual animal, $HgCl_2 \pm$ MOPC-21 *versus* $HgCl_2 + OX8$, $P = 0{\cdot}001$ and 0·0001 in experiments 1 and 2, respectively). The pattern of rise in experiment 2 is illustrated in Fig. 5.

DISCUSSION

In $HgCl_2$-induced autoimmunity in the BN rat, treatment with OX8 significantly reduces the severity but not the incidence of arthritis, has no such protective effect against caecal vasculitis, nor does it influence the production of IgG anti-collagen or MPO antibodies. Treatment with OX8 significantly enhances the $HgCl_2$-induced rise in total IgE at early time points (days 4–7) but does not affect the peak IgE concentration or subsequent autoregulation (days 12–18). There was no change in plasma IFN-γ concentration, within the detection limit of the ELISA, in animals treated with $HgCl_2 \pm MOPC\text{-}21$, but there was a rise in plasma IFN-γ in animals treated with $HgCl_2 + OX8$ between days 9 and 17. FACS analysis of peripheral blood cells confirmed that *in vivo* OX8 treatment had successfully depleted animals of $OX8^+$ lymphocytes, but not NK cells, at the start of and throughout each experiment. The percentage of $OX8^+$ lymphocytes in all groups rose towards the end of each experiment, but this did not influence the significant protective effect against arthritis.

Table 1. Incidence and severity of mercuric chloride ($HgCl_2$)-induced arthritis

	Incidence (%)	Median peak score	Median cumulative score
Experiment 1			
$HgCl_2 + PBS/MOPC\text{-}21$	80	7·5	24
$HgCl_2 + OX8$	90 NS	3 *$P = 0{\cdot}009$	6 *$P = 0{\cdot}005$
Experiment 2			
$HgCl_2 + PBS/MOPC\text{-}21$	100	7·0	28·5
$HgCl_2 + OX8$	80 NS	4 *$P = 0{\cdot}009$	14·5 *$P = 0{\cdot}01$

* *Versus* $HgCl_2 + PBS/MOPC\text{-}21$, Mann–Whitney *U*-test.

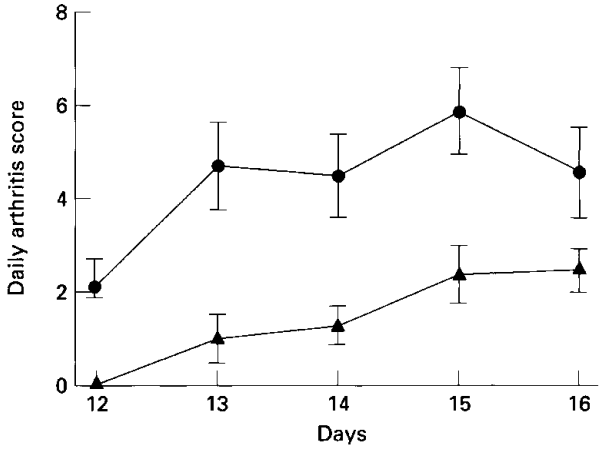

Fig. 2. Daily arthritis score in BN rats treated with mercuric chloride ($HgCl_2$) + PBS/MOPC-21 ($n = 10$, ●) and $HgCl_2$ + OX8 ($n = 10$, ▲) in experiment 1. Bars represent the mean scores, error bars the s.e.m.

In the $HgCl_2$ syndrome immunohistochemical staining of tissues has revealed an increase in $CD8^+$ cells at peak disease in ankle joints, caecum, small bowel and lung [4,12]. The proportion of $CD8^+$ lymphocytes also rises significantly in peripheral blood at 13 and 20 days [12], as seen at the end of the two experiments reported here. The functional significance of this accumulation is unclear, but a role in autoregulation is unlikely, as OX8 treatment did not effect immunoregulation of the total IgE, IgG anti-collagen, MPO and glomerular basement membrane (GBM) response, or proteinuria [10,11].

An immunosuppressive role for $CD8^+$ cells during the initiation of the syndrome is suggested by the observation that transfer of splenocytes from BN rats at peak $HgCl_2$ disease [5] or of pathogenic T cell clones [23] into naive BN rats will only induce the full autoimmune syndrome if the recipients have been treated with OX8. The authors [5] suggest that this indicates that the development of autoimmunity in these situations is dependent on

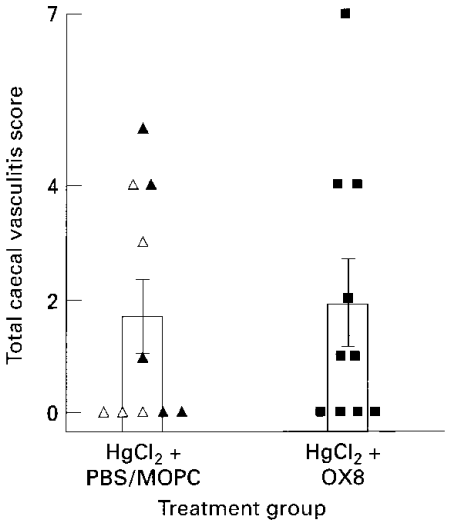

Fig. 3. Total caecal vasculitis scores at day 16 in BN rats treated with mercuric chloride ($HgCl_2$) + PBS/MOPC-21 and $HgCl_2$ + OX8 in experiment 1. Bars represent the mean scores, error bars the s.e.m. △ ($HgCl_2$ + PBS), ▲ ($HgCl_2$ + MOPC-21), and ■ ($HgCl_2$ + OX8) represent the individual scores for each animal.

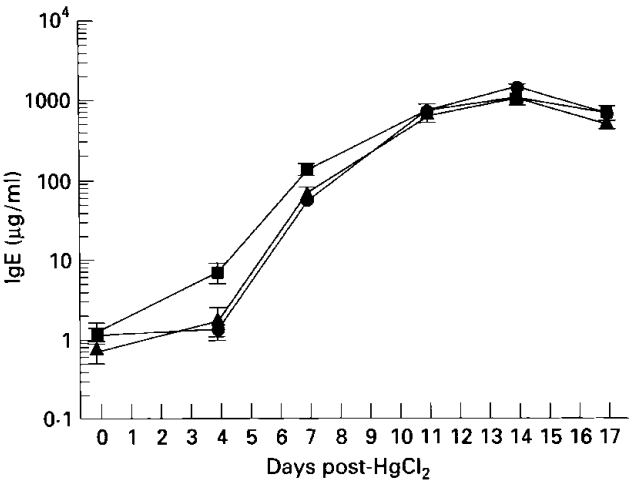

Fig. 4. The mean plasma total IgE concentration in BN rats treated with mercuric chloride ($HgCl_2$) + PBS ($n = 10$, ●), $HgCl_2$ + MOPC-21 ($n = 10$, ▲) and $HgCl_2$ + OX8 ($n = 10$, ■) in experiment 2. Error bars show the s.e.m.

both autoreactive T cells and an inhibition of immunosuppressive $CD8^+$ cells. If this is the case it might be predicted that OX8 treatment would exacerbate the syndrome in $HgCl_2$-treated naive animals. This has not been found and, instead, the titre of anti-GBM antibodies and the severity of proteinuria was suppressed in $HgCl_2$ + OX8-treated animals [10,11]. These observations contrast with the transfer experiments and suggest that in this situation $CD8^+$ cells may have a proinflammatory effect in the initiation phase of the syndrome.

The finding of a significant reduction in the severity of arthritis, but not vasculitis, in BN rats treated with $HgCl_2$ + OX8 also suggests that a population of $CD8^+$ cells may act in a direct proinflammatory capacity with respect to the pathogenesis of $HgCl_2$ A, but that such cells are not involved, at least to such a significant extent, in the pathogenesis of caecal vasculitis. $HgCl_2$ A and vasculitis have both been shown to be α/β T cell-dependent [4,7]. If there are both directly proinflammatory $CD4^+$ and $CD8^+$ T cells in this α/β T cell population, it would not be surprising to find a

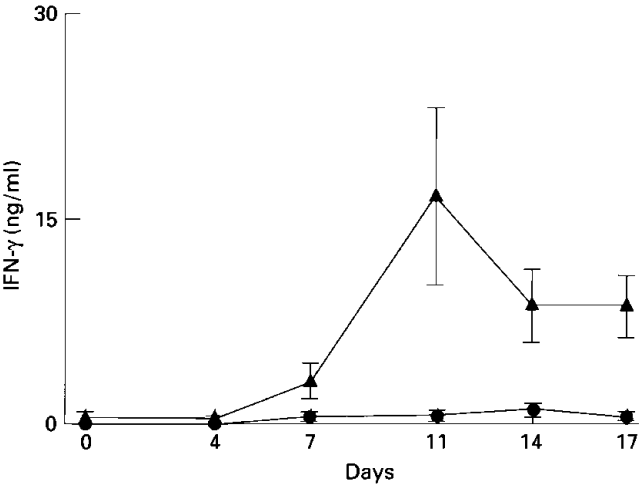

Fig. 5. The mean plasma IFN-γ concentration in BN rats treated with mercuric chloride ($HgCl_2$) + PBS/MOPC-21 ($n = 10$, ●) and $HgCl_2$ + OX8 ($n = 10$, ▲) in experiment 2. Error bars show the s.e.m.

partial protection if either population were removed, assuming that neither is redundant. This prediction is supported by our findings in the joint but not the caecum. The lack of response to OX8 in the caecum may be because an inflamed site here is exposed to other non-specific proinflammatory agents, such as bacteria, which may provide a strong secondary stimulus to inflammation, making the role of a particular cellular population, such as $CD8^+$ cells, less important. This interpretation is supported by the finding that pre-treatment of BN rats with broad spectrum anti-microbials reduces the severity of vasculitis in this model [3].

Support for the hypothesis that a directly proinflammatory population of $CD8^+$ cells may exist comes from the description in humans and rats of distinct subsets of $CD8^+$ T lymphocytes, distinguished by the profile of cytokines which they produce [24,25]. The secretion of cytokines by these subsets is reported to be regulated by the cytokine environment in a similarly precise (but different) manner to the control of Th1 and Th2 secretion. One prediction from these *in vitro* findings is that rat $CD8^+$ cells have the potential to differentiate *in vivo* into different populations, with differing profiles of cytokine secretion and differing functions. This is supported in the $HgCl_2$ model, where OX8 treatment (i) protects against arthritis, suggesting a proinflammatory $CD8^+$ subset; and (ii) exacerbates both transfer of disease and susceptibility to rechallenge, suggesting a separate anti-inflammatory or regulatory subset. The FACS analysis of peripheral blood cells suggests that the *in vivo* protocol of OX8 treatment depleted $CD8^+$ T cells but did not affect the NK cell population. This raises the possibility that the putative proinflammatory arthritogenic CD8 subset may be either the α/β or γ/δ T cell.

Evidence for separate effector and regulatory CD8 populations is found in other models of autoimmune disease. In experimental allergic encephalomyelitis (EAE) in mice CD8-/- animals develop milder acute disease with lower mortality, but are more susceptible to relapse in the chronic phase [19], suggesting two functionally different populations of $CD8^+$ cells, one with proinflammatory actions involved in the acute phase and one with regulatory actions in the chronic phase. A proinflammatory role for $CD8^+$ cells in pathogenesis is suggested in murine autoimmune thyroiditis, myosin-induced myocarditis, and in experimental autoimmune myasthenia gravis where CD8 depletion *in vivo* results in protection from disease [13,14,17]. A similar proinflammatory role is suggested in diabetes by the absolute requirement for $CD8^+$ cells in splenocyte transfer of disease to non-obese diabetic (NOD) mice [15] and by the finding that OX8 treatment inhibits the development of diabetes in BB [20] and lymphopenic rats [16].

An alternative explanation for the protective action on $HgCl_2$ A is that OX8 treatment has released a separate anti-inflammatory population from negative control by $CD8^+$ cells. Support for a tonic control over Th1 and Th2 cells comes from the report that removal of $CD8^+$ cells from rat splenocyte culture results in increased IL-4 mRNA expression and IFN-γ secretion by $CD4^+$ T cells [26]. The exacerbation of the total IgE response and a rise in serum IFN-γ in the $HgCl_2$ + OX8-treated animals may indicate a similar effect *in vivo*. As $HgCl_2$ induces a Th2 response [1,8,9], it is perhaps not surprising that release of Th cells from inhibitory CD8 control might initially result in an exacerbation of Th2 responses (IgE at days 4–7), followed by an increase in Th1 responses (IFN-γ at days 9–17), particularly as this matches the time that Th1 responses have been found to recover in this model [27]. Furthermore, the known inhibitory effect of IFN-γ on IgE production [28] may explain why the enhancement in IgE was not found after day 7. As $HgCl_2$ A is α/β T cell-dependent and Th2-associated, it is possible that an enhancement of regulatory Th1 functions (IFN-γ at days 9–17) might have resulted in the protective effect on the arthritis. This could be clarified by studying the effect of IFN-γ treatment on arthritis in this model.

Alternatively, the protective effect of OX8 on arthritis may have resulted from the release of a separate non-Th cell anti-inflammatory population. One candidate is the NK cell population, which was not numerically affected by this treatment protocol, and is a potential source of the increased concentration of plasma IFN-γ (review in [29]). This might be clarified if it were possible to target the rat NK cell population *in vivo*, or if the protective effect against arthritis were lost following *in vivo* treatment with the concentration of OX8 that is known to inhibit NK cell function.

Data describing the role of $CD8^+$ cells in the pathogenesis of inflammatory arthritis in other animal models are contradictory. In adjuvant arthritis peripheral blood $CD8^+$ lymphocytes rise and correlate significantly with the degree of inflammation in individual animals [30] and are present in synovial tissues [31], but OX8 treatment is reported to have no effect either on the development of arthritis [32,33] or on established disease [34]. The resistant Buffalo strain is reported to have a significantly lower CD4/CD8 ratio in blood, spleen and lymph node compared with the susceptible Holtzman strain, and selective depletion of $CD8^+$ cells (with low dose cyclophosphamide) in Buffalo rats induces AA, suggesting an anti-inflammatory role for $CD8^+$ cells in this strain [35]. In collagen-induced arthritis, although $CD8^+$ cells are found to accumulate in rat synovial sections, anti-CD8 therapy has no effect on established arthritis in mice [36,37] but reduces the severity of arthritis in castrated female rats, suggesting a pro-inflammatory role in this instance [38].

The data reported here provide evidence that $CD8^+$ cells are capable, either directly or indirectly, of a proinflammatory or effector role in the pathogenesis of inflammatory arthritis in the rat. These results also provide the first direct indication that $CD8^+$ cells may be involved in such a capacity in the initiation phase of $HgCl_2$-induced autoimmunity, and this supports similar findings in other animal models of autoimmune disease.

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