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Photodynamic therapy; a comparison with other immunomodulatory treatments of adjuvant-enhanced arthritis in MRL-lpr mice

L. G. RATKAY, R. K. CHOWDHARY*†, H. C. NEYNDORFF†, J. TONZETICH, J. D. WATERFIELD & J. G. LEVY*† *Department of Oral Biology, Faculty of Dentistry, *Department of Microbiology, University of British Columbia, and †Quadra Logic Inc., Vancouver, British Columbia, Canada*

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SUMMARY

Although numerous experimental immunomodulatory regimens have been reported to be effective in the treatment of rheumatoid arthritis, they also produce undesirable side effects. An alternative specific modality of localized treatment is photodynamic therapy (PDT). In this study we treated 13-week-old MRL-lpr mice whose spontaneous arthritis was enhanced by intradermal injection of Freund's complete adjuvant (FCA). One group received transcutaneous photodynamic therapy at days 0, 10, and 20, following the FCA injection. The other groups were injected with 1 mg/kg per day indomethacin, 40 mg/kg per day cyclosporin A (CsA), or treated with 3 Gy sublethal whole body irradiation (WBI). The development of swelling was monitored for 1 month, at which time proteinurea, lymphadenopathy and the histopathology of the joints and kidneys were assessed. The results demonstrated that PDT and the conventional treatments significantly ameliorated swelling of the hindlimbs from 70% in the untreated FCA-injected animals to below the 19% level characteristic of the unmanipulated control. Histological examination showed a reduction in pannus formation, and cartilage and bone destruction, the characteristics of adjuvant-enhanced arthritis. PDT did not affect the survival rate, lymphoproliferation, or proteinuria of the treated animals. However, indomethacin increased proteinurea, and was less effective in preventing cartilage and bone destruction. Furthermore, lower doses of CsA and WBI exacerbated arthritis activity. These results indicate that photodynamic therapy can inhibit the development of adjuvant-enhanced arthritis in MRL-lpr mice with similar effectiveness to the conventional treatments, but without their negative side effects.

Keywords photodynamic therapy experimental arthritis immunomodulation mice

INTRODUCTION

The MRL-MpJ-*lpr/lpr* (MRL-lpr) mouse strain develops an early autoimmune disease, sharing similarities with both human systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The animals develop skin lesions, immune complex-mediated glomerulonephritis, lymphoproliferation, and inflammatory arthritis [1,2]. Serologically the disease can be characterized by anti-nuclear antibodies, rheumatoid factor production and antibodies against extracellular matrix proteins [3,4].

The arthritic component of the disease in this mouse strain has become less severe in the past 5 years, due probably to breeding programmes that select for enhancing the SLE-like component of the disease. In order to enhance the RA features of the disease we have developed a model utilizing Freund's complete adjuvant (FCA). Animals injected intradermally with FCA demonstrate both an accelerated and enhanced form of the spontaneously occurring RA-like disease [5].

Correspondence: Dr L. G. Ratkay, Department of Oral Biology, Faculty of Dentistry, 2199 Wesbrook Mall, University of British Columbia, Vancouver, B.C. V6T 1Z3, Canada.

SLE has been treated or prevented in MRL-lpr mice by a number of experimental regimens; total lymphoid irradiation [6], neonatal thymectomy [7], cyclosporin A (CsA) [8,9], and MoAb therapy against the pan T cell marker Thy-1 [10,11], or the T helper cell determinant CD4 [12–15]. However, similar treatments on the spontaneous arthritis of these mice have been less frequently studied [16,17] as the mice have a 50% mortality rate at 5·5 months and do not develop a severe form of the disease. Our enhanced model of RA in MRL-lpr mice will prove useful in studying both the prevention and therapeutic intervention in the murine disease.

In a new form of RA therapy Holoshitz *et al.* demonstrated that inactivated T cell clones are capable of ameliorating transfer adjuvant arthritis [18]. A modification of this technique using photoinactivated autoimmune splenocytes has been reported by Berger *et al.* [19]. In this latter instance psoralen and ultraviolet A light (8-MOP-UVA or PUVA)-treated syngeneic lymphocytes prevented autoimmune disease after transfer to MRL-lpr mice. Photoactivated 8-MOP, besides its effect of crosslinking DNA, probably fixes the T cell receptor on the cell surface making its idiotype more immunogenic. Thus the 'fixed'

T cell clones provide an effective 'vaccine' that functions by controlling the effector clones responsible for the disease.

Benzoporphyrin derivative monoacid ring A (BPD) is a second generation photosensitizer [20,21] currently being used in clinical trials for photodynamic therapy (PDT) [22] of malignant skin lesions [23] and psoriasis [24]. BPD is a hydrophobic chlorin-like porphyrin derivative. It appears to be cleared rapidly from tissues, thus reducing the duration of normal skin photosensitivity [25], a concern with first generation photosensitizers. BPD is activated by 690-nm red light, a wavelength which penetrates tissue better than the wavelength used for first generation photosensitizers and is not attenuated by haemoglobin. Studies with this photosensitizer have shown that it is taken up selectively by activated lymphocytes (those bearing either the IL-2 receptor (IL-2R) or high levels of HLA-DR antigen) or other highly proliferative cells, thus rendering these populations susceptible to photodynamic destruction with 690-nm light [26]. Further, in recent experiments we demonstrated that BPD could be activated transdermally while in the circulation by exposure of animals to whole body illumination with red light [27]. These treatments resulted in photoactivation of between 50% and 70% of circulating BPD, and caused no skin photosensitivity, because treatment was initiated at a time when blood levels of BPD were high, and accumulation in the skin had not yet taken place. These observations led to the experiments reported here, in which we attempted to determine whether transdermal light treatment of BPD-injected MRL-lpr mice could ameliorate the development of adjuvant enhanced arthritis, presumably via selective photodynamic destruction of activated leucocytes in the circulation.

MATERIALS AND METHODS

Animals

MRL-MpJ-*lpr*/*lpr* (MRL-lpr) mice were obtained from a breeding colony located in the animal facilities in the Department of Oral Biology, University of British Columbia. This colony was established from stocks originally purchased from the Jackson Laboratories (Bar Harbor, ME) and bred for 2–9 generations. Histological analysis of different generations of these mice demonstrated no difference in the arthritic pathology of the spontaneous arthritis or in the ability of FCA to enhance the disease (data not shown). The mice were maintained on a standard diet with water *ad libitum*. The colony was routinely tested for *Mycoplasma pulmonis*, and *Myc. arthritidis*, rodent coronaviruses (including hepatitis), and Sendai virus using Murine ImmunoComb test (Charles River Laboratories, Wilmington, MA). Only antibodies against coronaviruses were detected.

Arthritis induction

MRL-lpr mice, 13–15 weeks old, were injected intradermally at two thoracic sites with each 0·05 ml Freund's adjuvant supplemented to 10 mg/ml with heat-inactivated, lyophilized *Mycobacteria tuberculosis* (RA37; Difco Labs, Detroit, MI).

Clinical evaluation

Mice were examined blind by an observer for clinical signs of arthritis every second day for 30 days after injection. The hind legs of the mice were measured with a micrometer, and joint swelling was recorded as the increase in bimalleolar ankle width. The presence of clinical disease was established when erythema and swelling were visually detectable and the bimalleolar ankle width increase was at least 0·15 mm (a range established from internal examiner standardization).

On day 30, the experiment was terminated. The urinary protein level was determined semiquantitatively by a colorimetric reaction using Albustix sticks (Miles, Ontario, Canada). Protein was measured as g/*l* with concentrations above 0·3 g/*l* considered abnormal. Lymphadenopathy was also evaluated at this time by a subjective 0–4 non-invasive scoring system based on palpation of the lymph nodes, with 0, no palpable lymph nodes; 1, one palpable lymph node in one area; 2, seriously enlarged lymph node(s) in one area; 3, seriously enlarged lymph node(s) in both axillary and inguinal areas; and 4, massively enlarged lymph nodes in all areas.

Histological evaluation

After sacrificing the animals, the joints and kidneys were dissected and fixed in 10% formalin. The joints were then decalcified in 10% formic acid for 48 h and both tissues processed for paraffin embedding. Serial 3–5 μm thick sections were stained with haematoxylin and eosin.

Histopathological alterations of the joints were evaluated by an observer, blinded to the experimental design, in consultation with two pathologists after standardization of the measurements. A minimum of 10 sections per joint were assessed for the presence of the following features: (i) subsynovial inflammation; (ii) synovial hyperplasia; (iii) cartilage erosion and pannus formation; and (iv) bone destruction. Within each parameter scores were assigned from 0 to 2 (0, normal; 1, mild; and 2, severe involvement).

Similarly, kidney sections were also examined for glomerular mesangial cell proliferation, crescent formation, interstitial cellular infiltration, and vasculitis. The histological changes were evaluated by a scoring system from 0 to 4, where 4 was considered severe, 3 as moderate disease, 2 corresponded to mild and 1 to minimal formed lesions.

Treatment regimens

Indomethacin (Sigma) was administered 1 mg/kg per day subcutaneously. CsA (a gift of Dr J. F. Borel, Sandoz Ltd., Canada) 40 mg/kg per day was injected intraperitoneally after dissolving it in olive oil by heating in a waterbath to 65°C. Whole body irradiation (WBI) was delivered as 3 Gy from a ^{60}cobalt source (Gammacell 220, Atomic Energy of Canada Limited, Ottawa, Canada) on day 1 of arthritis induction. Liposomally formulated benzoporphyrin derivative monoacid ring A (BPD) was obtained from Quadra Logic Technologies (Vancouver, Canada). Aliquots were made up in 5% dextrose in water and 0·5 mg/kg body weight BPD was injected intravenously. Whole body transcutaneous photodynamic therapy was administered to unshaved mice with 80 J/cm^2 light dose applied on day 0, 10, and 20 after arthritis induction. The optimal concentration of BPD and light dose necessary for cell killing was determined in preliminary studies [27].

Statistical analysis

Data are expressed as mean $\pm$ s.e.m. Intergroup differences were established by ANOVA and Bonferroni tests.

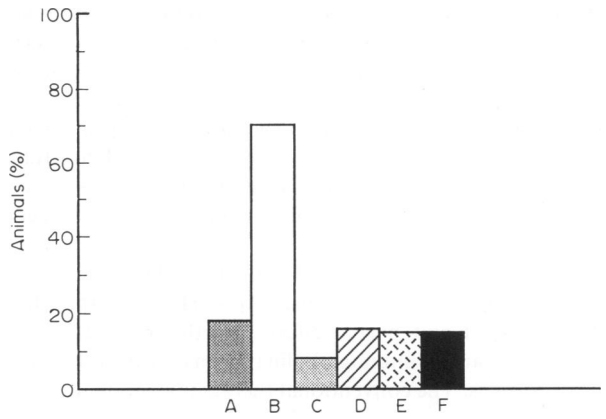

Fig. 1. Animals displaying swelling and erythema of hindpaws. A, Spontaneous ($n=28$); B, Freund's complete adjuvant (FCA) ($n=66$); C, FCA + indomethacin ($n=12$); D, FCA + cyclosporin A (CsA) ($n=21$); E, FCA + whole body irradiation (WBI) ($n=34$); F, FCA + photodynamic therapy (PDT) ($n=33$).

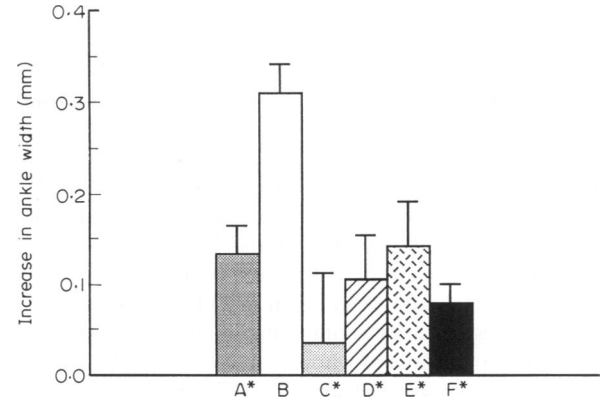

Fig. 2. Bimaleolar ankle width 30 days after Freund's complete adjuvant (FCA) injection. A, Spontaneous ($n=28$); B, FCA ($n=66$); C, FCA + indomethacin ($n=10$); D, FCA + cyclosporin A (CsA) ($n=12$); E, FCA + whole body irradiation (WBI) ($n=34$); F, FCA + photodynamic therapy (PDT) ($n=33$). *Corresponding value significantly different from the FCA group's value ($P<0.01$ ANOVA and Bonferroni multiple comparison test).

RESULTS

Effect of treatments on clinical signs of arthritis

MRL-lpr mice, randomly assigned into a treatment group, were monitored for 30 days for clinical signs of arthritis (Fig. 1). Seventy per cent (46/66) of FCA-injected control animals developed erythema and swelling during the experiment. This is in agreement with our previous findings [5]. The incidence of swelling was reduced in the treatment groups below the level found with the non-injected control animals (18%, 5/28). In the indomethacin group a somewhat lower percentage of animals developed the swelling (8%, 1/12). Similarly all treatment groups had a significantly reduced maximal increase in ankle width (Fig. 2).

Effect of treatments on histopathology of the joints

Thirty days after adjuvant injection histological evaluation of the joints was carried out for the various immunomodulatory

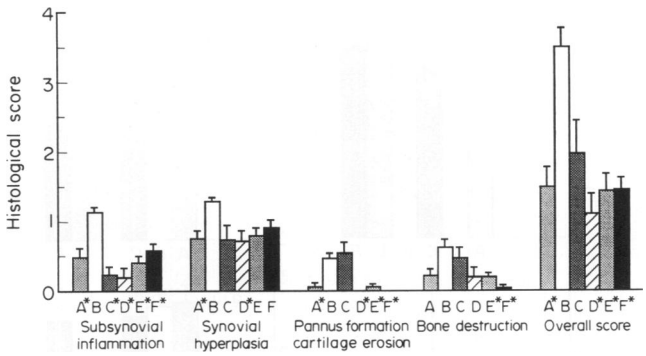

Fig. 3. Histopathological evaluation of the joints 30 days after Freund's complete adjuvant (FCA) injection. The histological scores were determined as described in Materials and Methods. A, Spontaneous ($n=18$); B, FCA ($n=58$); C, FCA + indomethacin ($n=12$); D, FCA + cyclosporin A (CsA) ($n=21$); E, FCA + whole body irradiation (WBI) ($n=32$); F, FCA + photodynamic therapy (PDT) ($n=27$). *Corresponding value significantly different from the FCA group's value ($P<0.01$ ANOVA and Bonferroni multiple comparison test).

treatments (Fig. 3). The analysis showed that all treatment groups exhibited significantly less subsynovial inflammation than the FCA-injected control. However, exacerbation of the synovial hyperplasia was notably prevented only by CsA. With the exception of indomethacin, all treatments effectively prevented significant pannus formation and cartilage erosion, which is characteristic of the FCA-enhanced arthritis in MRL-lpr mice. Bone destruction was prevented only by WBI and PDT. The overall histological scores summarize the prophylactic effects of the described therapeutic modalities. All treatments, except indomethacin, significantly prevented the exacerbation of the disease; indomethacin exerted a significant effect only on subsynovial inflammation.

Effect of treatments on systemic disease parameters

As the spontaneous arthritis is an integral component of the underlying SLE-like disease, it was of interest to evaluate the systemic effects of the treatments.

The survival of mice from the different treatment groups was compared with the FCA-injected control (Fig. 4a). The mortality rate of the groups was approximately 22% (50/230), which is characteristic of this mouse strain at 4 months of age [3]. The weight gain during the 30 days experimental period was slightly reduced in the WBI group, but the differences were not significant (Fig. 4b). Similarly, proteinuria measured at day 30 after FCA injection was not significantly different, although the value for the indomethacin group was apparently elevated (Fig. 4c). This might reflect deteriorating kidney condition that is not evident at the histological level in individual animals as the consequence of indomethacin treatment.

It was noteworthy that the lymphadenopathy was significantly prevented only by WBI (Fig. 4d).

Histopathological evaluation of the kidneys indicated there were no significant changes between the various treated animals and the untreated controls (Fig. 5).

DISCUSSION

Human rheumatoid arthritis is a complex inflammatory disease mediated by T lymphocytes that infiltrate the synovial mem-

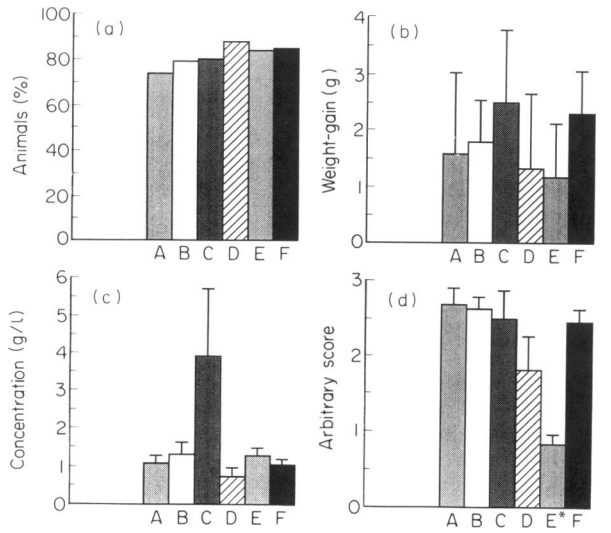

Fig. 4. Evaluation of systemic changes 30 days after Freund's complete adjuvant (FCA) injection. (a) Survival. (b) Weight gain. (c) Proteinurea. (d) Lymphadenopathy. A, Spontaneous ($n=28$); B, FCA ($n=66$); C, FCA + indomethacin ($n=10$); D, FCA + cyclosporin A (CsA) ($n=12$); E, FCA + whole body irradiation (WBI) ($n=34$); F, FCA + photodynamic therapy (PDT) ($n=33$). *Corresponding value is significantly different from the FCA group's value ($P<0.01$ ANOVA and Bonferroni multiple comparison test).

brane (reviewed in [28,29]). Two animal models that have been used to mimic the human disease process are the adjuvant arthritis seen in rats [30] and the mild spontaneous arthritis seen in MRL-lpr mice [15]. In the latter system we have demonstrated that the incidence of disease and its severity can be accelerated by i.d. injection of FCA supplemented with *Myco. tuberculosis* [5] that induces a pathology more consistent with that seen in patients.

Therapeutic interventions in RA are primarily either anti-inflammatory or immunosuppressive; the latter designed to target the CD4+ regulatory T cells that are central to the disease process. Such pharmacologic and physical agents that have been successfully used in either animal models or the human disorder are indomethacin, prednisolone, cyclophosphamide, CsA, thoracic duct drainage [31], lymphocytapheresis [32], and whole body irradiation (total lymphoid irradiation [33]). Unfortunately these treatment procedures have systemic effects on cell populations other than those directly involved in the pathogenic mechanism(s).

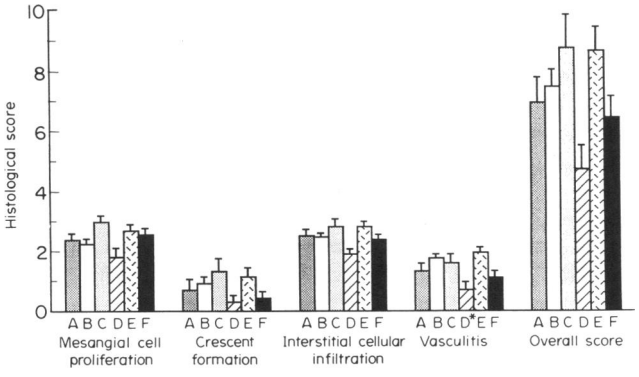

Fig. 5. Histological evaluation of the kidneys. The histological scores were established as described in Materials and Methods. A, Spontaneous ($n=16$); B, Freund's complete adjuvant (FCA) ($n=45$); C, FCA + indomethacin ($n=10$); D, FCA + cyclosporin A (CsA) ($n=10$); E, FCA + whole body irradiation (WBI) ($n=26$); F, FCA + photodynamic therapy (PDT) ($n=18$). *Corresponding value is significantly different from the FCA group's value ($P<0.05$ ANOVA and Bonferroni multiple comparison test).

MoAbs against pan T cell antigens, CD4, and T cell activation antigens are under clinical trials. However, they show mostly transient and partial effects. Furthermore, there are problems with anti-immunoglobulin response against the administered MoAbs. The conventional therapeutic modalities used in these experiments all exhibited beneficial effects on animals receiving adjuvant enhancement. Indomethacin (a non-steroidal anti-inflammatory agent (NSAID) which inhibits the synthesis of prostaglandins in inflamed tissues), while it significantly prevented swelling and subsynovial inflammation, increased the proteinuria and failed to prevent cartilage and bone destruction. CsA, a T cell-specific immunosuppressive antirheumatic drug, selectively inhibits T helper/inducer function [34]. It has been effectively used in RA therapy, but its possible side effects of nephrotoxicity and tumorogenicity preclude it from general use [35]. Sublethal whole body irradiation, or the more frequently used total lymphoid irradiation therapy, has a non-specific immunosuppressant effect by its elimination of dividing cells. Again, its practical application in rheumatology is limited because of its side effects. CsA and WBI inhibited both the clinical induction of disease and the histopathology associated with the joints. However, the overall toxicity of CsA and WBI as reflected by renal function and histology, survival, and weight gain was minimal in our study. In addition these latter two immunomodulators are highly dose-dependent; a single dose of whole body irradiation (1·5 Gy) not only failed to prevent the arthritis (57%, 8/14 incidence of swelling), but in some cases paradoxically it exacerbated the disease, similar to a one time response at day 0 treatment of CsA (67%, 14/21 incidence of swelling).

The animals treated by transdermal PDT with BPD showed no signs of skin photosensitivity following treatment. However, this treatment produced a significant alteration in arthritis development, in that it prevented the clinical and histopathological signs of adjuvant-enhanced arthritis, and was especially effective in preventing the more severe stages of disease (pannus formation, cartilage erosion, and bone destruction). In terms of general immune function (mitogen responses), liver enzyme levels, and blood cell counts, treated animals were comparable to controls during a 7-day follow up (Chowdhary *et al.*, in preparation). The only effect noted by this transdermal treatment is a transient (during the first 18 h post-treatment) drop in circulating leucocytes. This has been observed in other mouse strains following transdermal PDT and does not appear to have any pathological significance (Richter *et al.*, unpublished data). In this study, the therapeutic dose had no effect on survival, weight gain, or proteinurea and histology of the kidney. It is not clear at this time whether this PDT effect on arthritis development resulted from a local elimination of activated cells in the region of the hind feet, or from the elimination of activated cells in the circulation. Ongoing studies suggest that the treatment may have both a local and a systemic effect, since pharmacoki-

netic analyses of blood from treated animals demonstrated that a significant amount of circulating BPD had been light-activated during the treatment [36]. These aspects are being further investigated.

These results indicate that photodynamic therapy can inhibit the development of adjuvant-enhanced arthritis in MRL-lpr mice with similar effectiveness to conventional treatments, but without their deleterious side effects. The application of the photosensitizer BPD and light to eliminate activated cells responsible for the inflammatory reaction at the arthritic site or within the circulation may have significant clinical implications in the treatment of human rheumatoid arthritis.

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