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The effects of anti-inflammatory agents on the serology and arthritis of the MRL lpr/ lpr mouse

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Mice of the MRL/MpJ lpr/lpr (MRL/l) strain spontaneously develop an autoimmune connective tissue disease that shares immunological and histopathological features with Systemic Lupus Erythematosus and rheumatoid arthritis [1]. To evaluate the usability of the MRL/l mouse strain for detecting anti-arthritic drugs we have studied the effect of various classes of compounds upon six disease parameters, namely: survival, lymphoproliferation (spleen weight), arthritis (histology), serum levels of the acute-phase reactant Serum Amyloid P component (SAP), the anti dsDNA antibodies (Ab) and the IgG + IgM rheumatoid factors (RF).

Material and methods

The MRL/l mouse strain, originally obtained from the Jackson Laboratories, was reared thereafter in the Ciba-Geigy breeding unit. The compound or the corresponding vehicle solution were given to the mice (8 to 10 mice/group) from 4 weeks of age to 24 weeks (female) or 28 weeks (male) of age. Unless otherwise specified the following drugs were administred for 5 out of 7 days via the oral route: D-*Penicillamine* (15 and 25 mg/kg/day), *Chloroquine* (10 and 30 mg/kg/day), *Gold (Sodium aurothiomalate)* (2.5 mg/kg/once a week and 10 mg/kg/twice a week, i.m.), *Suramin* (20 and 50 mg/kg/once a week, i.v.), *Prednisolone* (0.6 and 6 mg/kg/day), *Cyclophosphamide* (5 and 10 mg/kg/day, i.p.), *Retinoic acid* (2.5 and 12.5 mg/kg/day, i.p.), *Cyclosporin A* (25 mg/kg/day, s.c. and 75 mg/kg/day), *Deoxyguanosine* (2.5 and 25 mg/kg/day), *Indomethacin and Piroxicam* (0.5 mg/kg/day), *Benoxaprofen* (40 mg/kg/day). D-Penicillamine and Deoxyguanosine were obtained from Fluka, Sodium aurothiomalate from Byk Gulden, Suramin from Bayer, Chloroquine from Sigma, Cyclophosphamide from Serva, Benoxaprofen from Lilly and the remaining compounds from internal sources.

SAP, anti dsDNA Ab and RF levels were measured by a solid phase Elisa [2], every 4 weeks beginning from 7 weeks of age. Mice were killed at 24 weeks of age (female) or at 28 weeks of age (male), spleen and body weights were recorded. The right hindpaws were excised and prepared for light microscopy. The histopathological evaluation was performed blind and randomly, scoring intensity of the arthritic changes on a 0 to 4 scale. Series of a minimum of 15 sections per hind paw were evaluated histologically. Statistical evaluation of the drug effects was done with the Mann-Whitney U test (1 sided) and considered significant if $p < 0.05$.

Results

In male mice, Chloroquine, Gold, Prednisolone, Cyclophosphamide, Benoxaprofen and Suramin given prophylactically significantly inhibited the rise in SAP and anti dsDNA Ab levels. This inhibitory effect was already detectable after 3 weeks of treatment and reached a maximum after

11 to 15 weeks of therapy. Female mice were less responsive to therapy, only Suramin and Cyclophosphamide inhibited the SAP levels and only Suramin and Prednisolone those of anti dsDNA Ab. Under the protocol used, D-Penicillamine, Cyclosporin A, Deoxyguanosine, Retinoic acid, Indomethacin and Piroxicam did not inhibit the rise of SAP and anti dsDNA Ab. All drugs tested inhibited, to some extent the RF levels. Cyclophosphamide was the only drug able to completely supress the lymphoproliferation. Spleen weight/body weight ratio similar to those observed in the non lpr bearing congenic MRL +/+ strain were observed with this drug. A partial control of the proliferative process was also observed in Cyclosporin A, Retinoic acid and Gold treated mice. Improvement in the cumulative frequency distribution of mortality was specially observed in female mice treated with Suramin and high doses of Chloroquine, Prednisolone and Cyclophosphamide, however the effect never reached a statistically significant level. Histologically the arthritis was significantly inhibited by Cyclophosphamide. Mean arthritis scores of 1.22 and 1.03 were scored in female and male mice treated with 10 mg/kg/day Cyclophosphamide respectively, while corresponding vehicle treated mice had mean scores of 3.26 (female) and 1.8 (male). Inhibition of joint inflammation was also found in mice of both sexes treated with Suramin and in male mice receiving Gold. D-Penicillamine, Chloroquine (only female data available), Cyclosporin A, Retinoic acid, Prednisolone, Deoxyguanosine, Indomethacin and Piroxicam did not inhibit the arthritis.

Discussion

These data suggest that the MRL/l mouse strain is suitable for the detection of anti arthritic drugs. Noteworthy is the fact that agents not or poorly profiled in classical animal models of inflammation, such as Chloroquine and Gold, could favourably influence several disease parameters. On the other hand the model does not seem suitable for detecting classical cyclooxygenase inhibitors. Whether dual cyclo/lipoxygenase or lipoxygenase inhibitors, could be profiled in this model warrants further experimentation. The finding that Cyclophosphamide was the only drug supressing the lymphoproliferation and profoundly inhibiting the arthritis, implies that this proliferative process and its biological consequences play an important role in the pathogenesis of the MRL/l mouse arthritis.

References

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