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The use of the murine chronic graft Vs host (CGVH) disease, a model for systemic lupus erythematosus (SLE), for drug discovery

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Abstract

Studies were conducted to evaluate the use of a murine SLE-like disease for the discovery of novel drugs: This disease is the result of a chronic form of a graft vs host (GVH) reaction. Using prednisolone (Pr), cyclophosphamide (Cy), indomethacin (Indo), and the isoxazol derivative, HWA 486, we found that only Indo was ineffective in inhibiting the SLE symptoms. Interestingly, HWA 486, which did not display any immunosuppressive activity, restored the suppressed T-cell response to the same level as found in healthy mice. We feel that this murine model of SLE could be of value for discovering substances with novel antirheumatic, and/or immunomodulating activities.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that virtually affects any organ in the body and is characterized by the development of antibodies against certain types of self-antigens. The nature of this disease has been studied since a great many years using animal models in which this disorder develops spontaneously. Gleichmann et al. [1] have investigated a chronic form of a murine graft vs host (GVH) disease. Instead of developing a runt disease, as is the case in illness caused by acute GVH reactions, these animals develop symptoms very similar to SLE due to an abnormal T-B cell co-operation. These include lymphadenopathy, immune complex glomerulonephritis, and the formation of multiple autoantibodies. Here we report our findings on the effect of a steroid, prednisolone (Pr), an alkylating agent, cyclophosphamide (Cy), a prostaglandin inhibitor, indomethacin (Indo), and the novel isoxazol derivative, HWA 486 [2, 3] on the disease development in animals with this autoimmune disorder.

Materials and methods

Female (C57bl/6 × DBA/2)F1 (F1-mice) and female DBA/2 from Charles River Wiga (Sulzfeld) were used. For the induction of the CGVH-reaction, F1-mice received 70×10^6 cells from DBA/2-mice (splenocytes and thymocytes 1 : 1) through i.v. injection on day 1 and 7. Starting on the 17th day after the first cell transplantation, the animals were treated with either Cy, HWA 486, Pr or Indo as indicated in the table and figure. 10 weeks after disease induction the mice were bled, the kidneys taken and the spleens removed and pooled.
The determination of the proliferative response of lymphocytes to mitogen was conducted as we have described [2]. Immune complexes fixed *in vivo* along the basement membrane of the glomeruli were detected by immune fluorescence as described by Gleichmann et al. [4].

Results and discussion

The determination of proteinuria is a simple method to follow the development of glomerulonephritis. Therapy with 10 mg/kg/day and more of HWA 486 resulted in substantial inhibition of urinary protein levels. None of the mice treated with 30 mg/kg/d HWA 486 showed any signs of developing proteinuria. This was also true for the group in which 50 mg/kg Cy was applied twice weekly, although when this agent was administered at 8 mg/kg/d only slight reduction of protein levels was observed. Indo did not protect against proteinuria (see table).

The development of proteinuria is due to the formation of immune complexes in the glomeruli, which together with complement lead to glomeruli destruction and renal failure. We found that, with one exception, the number of glomeruli in which immune complex deposition was detected could be correlated with the amount of protein present. Those animals with proteinuria also had elevated depositions. In the urine of Cy treated (50 mg/kg/2× week) mice no elevated protein level was found. This corresponded to the lack of detectable immune complexes in their glomeruli (see table). The Pr treated mice were an exception. The proteinuria of those mice was lowered, but significant immune complex deposition was found. This may have to do with the mode of action of this agent.

In order to further evaluate the effects of agents on the CGVH disease, the CGVH-index was used. This index is defined as the relationship between spleen and body weights of healthy untreated mice with those same weights of diseased mice (see table). Cy, Pr and HWA 486 reduced this index, whereas Indo further increased it.

The ability of lymphocytes to be stimulated by mitogens is significantly changed in mice undergoing the CGVH-disease. The concanavalin A (Con A), phytohemagglutinin (PHA), and pokeweed mitogen (PWM) induced stimulation of lymphocytes was significantly suppressed, whereas the T-cell independent B-cell response to dextran sulfate (DXS) was enhanced. Treatment with HWA 486 restored the respone of lymphocytes to Con A and PMW, and improved the activity to PHA. Depending on the dose, Cy partially restored or suppressed the mitogen induced responses (see figure).

Our studies were conducted to evaluate the use of the murine CGVH-model for discovery of novel drugs. The CGVH-disease differs greatly from the acute GVH-disorder in that the mice display symptoms very similar to those of SLE. Although this model of SLE will never replace animal models in which SLE spontaneously develops, we do see some advantages with this model concerning drug discovery, e.g. healthy animals are used, avoiding expensive breeding facilities with specific pathogen free conditions (a major problem

Table 1
Effects of drugs on the CGVH–disease.

Drug	Dose mg/kg/d	Survival rate %	GVH-Index change in %	Immune complex deposition %-inhibition	Proteinuria mg/dl
None	–	75	0.0	0	300
HWA 486	5	95	– 5.5	4	100
HWA 486	10	100	– 8.5	0	70
HWA 486	20	100	– 36.8	28	10
HWA 486	28	100	– 77.7	92	10
Cyclophos.	8	100	– 97.2	5	100
Cyclophos. *	14	100	– 76.5	5	200
Cyclophos. *	28	100	– 82.5	70	40
Cyclophos. *	50	80	– 87.3	100	10
Indomethacin	1	100	+ 121.8	4	250
Prednisolone	2	100	– 75.5	6	10

* mg/kg/2×week.

$$\text{CGVH index} = \frac{\text{weight of spleens X/weight of mice X}}{\text{spleen weight of healthy mice / weight of healthy mice}}$$

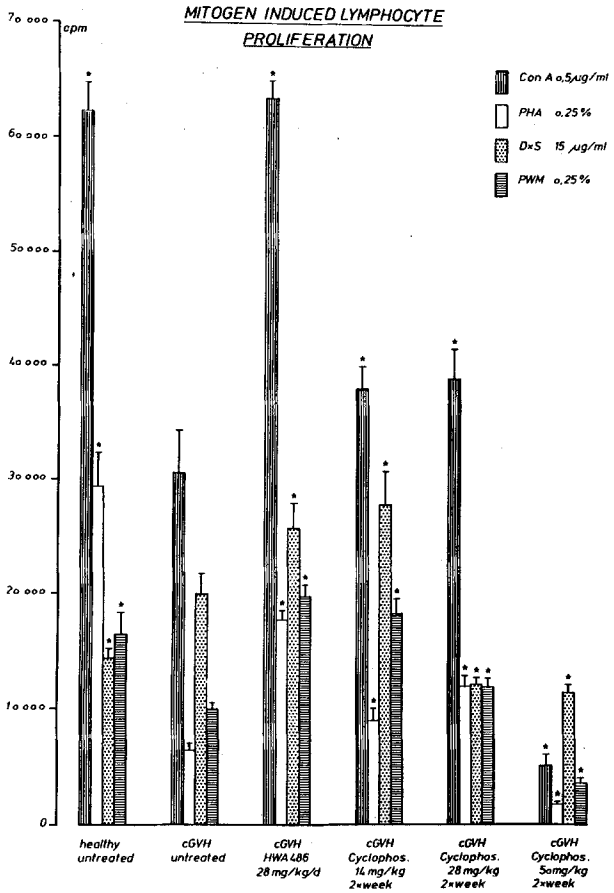

Figure 1
The splenic lymphocytes, isolated from F1-mice (10 weeks after initiation of disease), were cultured together with the above listed mitogens. After 60 h, the cells were given 0.25 µCi of (^{3}H)-thymidine, and incubated for another 8 h before harvisting. $^*p < 0.05$.

with autoimmune MRL/1-mice); the disease can be initiated at a time convenient for the investigator; the disease progression follows a known pattern and, thus, can be manipulated at any desired time point of its development.

Concerning drug testing, we found that therapy of these mice with Pr, Cy, or HWA 486 lead to inhibition of SLE symptoms, whereas Indo was ineffective. According to the dosing schedule, Cy was either suppressive or had restoring activity on the immune response. Interestingly, the novel isoxazol derivative HWA 486, which did not display any immunosuppressive activity, restored the immune response of these diseased animals to that of healthy mice. Although further testing of drugs should be conducted, we feel that this murine model of SLE could be of value for discovering substances with novel antirheumatic, and/or immunomodulating activities.

Literature

[1] E. Gleichmann, E. H. van Elven and J. P. W. van der Veen, *A systemic lupus erythematosus (SLE)-like disease in mice induced by abnormal T-B cell co-operation. Preferential formation of autoantibodies characteristic of SLE.* Eur. J. Immunol. *12*, 152–139 (1982).

[2] R. Bartlett and R. Schleyerbach, *Immunopharmacological profile of a novel isoxazol derivative HWA 486, with potential antirheumatic activity-I. Disease modifying action on adjuvant arthritis of the rat.* Int. J. Immunopharm. *7*, 7–18 (1985).

[3] R. Bartlett, *Immunopharmacological profile of HWA 486, a novel isoxazol derivative. II. In vivo immunomodulating effects differ from those of Cyclophosphamide, Prednisolone, or Cyclosporin A.* Int. J. Immunopharm. *8*, 199–204 (1986).

[4] E. H. van Elven, J. Agterberg, S. Sadal and E. Gleichmann, *Diseases caused by reactions of T lymphocytes to incompatible structures of the major histocompatibility complex II. Autoantibodies deposited along the basement membrane of skin and their relationship to immune-complex glomerulonephritis.* J. Immunol. *126*, 1684–1691 (1981).