Agents Actions *38*, *Special Conference Issue* (1993)

0065-4299/93/02C118-02 $1.50+0.20/0

Antibodies of IgG_2 subtype are involved in the immunological inflammatory response of TNBS-induced enteritis in rats

K. Auler[1], H. Beier[2] and N. Selve[1,*]

Depts. of Pharmacology[1] and Pharmacokinetics[2], Grünenthal GmbH, Zieglerstr. 6, 5100 Aachen, Germany

Abstract

Conclusive evidence of particular aetiologic factors in human inflammatory bowel diseases (IBDs) has not been obtained. However, there is information to suggest that immunologic mechanisms are involved in the pathogenesis and perpetuation of both ulcerative colitis and Crohn's disease. A TNBS-induced enteritis was developed by immunisation and local challenge of rats with TNBS. This animal model mimicked several features of human Crohn's disease. Because of the immunoglobulin component in human Crohn's disease, investigation of the TNBS-induced immunological response in the animal model was performed. A specific ELISA was developed to assess the humoral part of the TNBS sensitisation in the rat. Investigation of sera obtained from sensitized animals 11 weeks after the start of TNBS immunisation procedure revealed a clear IgG-specific immune response against TNBS. Immunoglobulin subtypes were differentiated by goat anti-rat antibodies and results indicate a specific IgG_2 subtype response.

Introduction

Ulcerative colitis and Crohn's disease are inflammatory bowel diseases of unknown aetiology although there is evidence for an immunological component. The high levels of immunoglobulin A (IgA) compared with the lower levels of IgG in normal intestinal mucosa is strikingly shifted in favour of IgG in IBD [1]. Moreover, there seem to be differences between ulcerative colitis and Crohn's disease with regard to the IgG subclass [2]. TNBS-induced enteritis [3] is a new animal model of IBD in which histological and biochemical similarities to human disease have been demonstrated. As active sensitisation against the antigen is the basis of the model, characterisation of the specific immune response was investigated.

* Author for correspondence.

Materials and methods

Animal treatment

Rats ($n=20$) were sensitized and boostered by intradermal injection of TNBS dissolved in Freund's incomplete adjuvant together with ovalbumin as a carrier [3]. Sera of TNBS-sensitized animals were collected 24 h before the first injection and then at 11 weeks. Sera of 11 additional representative experimental animals were pooled.

TNBS antibody determination by ELISA

Non-specific binding sites of antigen-coated microwell plates were blocked with 3% bovine serum albumin (BSA) and 0.01% Tween 80 in PBS. For routine determination of anti-TNBS antibodies, we used BSA mixed with TNBS 1:16 (w/w). Samples were pre-diluted 1:50 and then sequentially diluted

Table 1
Anti-TNBS-IgG response (AUC ratios – see Methods) of 20 immunised rats before immunisation (O) and at 11 weeks after sensitisation (S).

No. 1		No. 2		No. 3		No. 4	
O	S	O	S	O	S	O	S
0.105	0.920	0.091	0.922	0.078	0.860	0.075	1.153
No. 5		No. 6		No. 7		No. 8	
O	S	O	S	O	S	O	S
0.102	1.020	0.106	0.953	0.090	1.025	0.078	0.888
No. 9		No. 10		No. 11		No. 12	
O	S	O	S	O	S	O	S
0.068	0.873	0.077	0.935	0.102	1.016	0.091	0.978
No. 13		No. 14		No. 15		No. 16	
O	S	O	S	O	S	O	S
0.067	0.850	0.065	0.814	0.069	0.924	0.077	0.867
No. 17		No. 18		No. 19		No. 20	
O	S	O	S	O	S	O	S
0.064	0.904	0.055	1.017	0.063	0.971	0.074	0.987

in 1:2 steps up to 1:6400 in coating buffer. Preincubated (5 min, 20 °C) samples were transferred to microwell plates and incubated at 4 °C overnight. Bound anti-TNBS antibodies were detected with anti-rat IgM or IgG, IgG_1 and IgG_{2a} specific peroxidase-labelled antibodies, using ABTS-H_2O_2 substrate for peroxidase activity determination.

Evaluation of titration curves

End-point dilutions were calculated as $\log_2$ values, range -5.644 (1:50) to -12.644 (1:6400). The area under the titration curve (AUC) was calculated using the trapezoidal rule. Standardisation of the test system was performed by co-determination of the pooled "standard" serum. Titration curves were related to this standard.

Results

Rats immunised with TNBS and ovalbumin (OA) showed a clear immunologic reaction to OA and TNBS–OA conjugate. For determination of TNBS-specific response, TNBS was bound to microtiter plates using BSA instead of OA. Cross-reactivity with BSA could be excluded and AUC values obtained were in the range of background values. AUC values of sera from 20 immunised rats at 11 weeks was 0.944 ± 0.018 (see Table 1). In contrast, sera from non-immunised rats did not show any response against TNBS–BSA conjugate (0.080 ± 0.034), these corresponding to background values. These results suggest our system may be a useful tool to determine specific TNBS-induced IgG response in rats. Antibody specifity was tested regarding IgM, IgG, IgG_1 and IgG_{2a}, revealing an IgG-specific immune response; this primarily being IgG_{2a}.

Discussion

This method has advantages over the formerly used skin test for characterising the type of immunological reaction [3]: it permits the quantification of the anti-TNBS immune response enabling reproducibility and accuracy of determining the immune response to TNBS to be assessed.
This method of determining anti-TNBS IgG immune response in immunised rats could also be used to select "standard" animals for the enteritis model i.e. those with minimum or maximum anti-TNBS titer.

References

[1] R. P. MacDermott, G. S. Nash, M. J. Bertovich, M. V. Seiden, M. J. Bragdon and M. G. Beale, *Alterations of IgM, IgG, and IgA synthesis and secretion by peripheral blood and intestinal mononuclear cells from patients with ulcerative colitis and Crohn's disease.* Gastroenterology *81*, 844–852 (1981).
[2] K. Kett, T. O. Rognum and P. Brandtzaeg, *Mucosal subclass distribution of immunoglobulin G-producing cells is different in ulcerative colitis and Crohn's disease of the colon.* Gastroenterology *93*, 919–924 (1987).
[3] N. Selve and T. Wöhrmann, *Intestinal inflammation in TNBS sensitized rats as a model of chronic inflammatory bowel disease.* Mediators of Inflammation *1*, 121–126 (1992).