

Treatment of multiple sclerosis with anti-measles cow colostrum

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Abstract. Previous virological and immunological studies have suggested that multiple sclerosis (MS) is an auto-immune disease triggered by a virus infection. In order to inhibit the growth of measles virus in the patient's jejunum, we obtained an IgA-rich cow colostrum containing anti-measles lactoglobulin resistant to proteases. This colostrum was orally administered to patients with MS to investigate its effect on the course of the disease.

Measles-positive antibody colostrum was orally administered every morning to 15 patients with MS at a daily dosage of 100 ml for 30 days. Similarly, measles-negative antibody (< 8) control colostrum was orally administered to 5 patients. As a clinical assessment, disability scores developed by the International Federation of Multiple Sclerosis Societies were used. As a result, of 7 high NT titre (512–5120) anti-measles colostrum recipients 5 patients improved and 2 remained unchanged. Among 8 low NT titre (8–32) anti-measles colostrum recipients 5 patients improved and 3 remained unchanged. However, of 5 negative NT titre (< 8) colostrum recipients 2 patients remained unchanged and 3 worsened. No side-effects were observed in colostrum recipients. These findings suggest the efficacy of orally administered anti-measles colostrum in improving the condition of MS patients ($P < 0.05$).

Introduction

Following the methods of Prasad et al. [7], we were able to rescue measles virus antigens from the jejunum of 7 of 11 multiple sclerosis (MS) patients in a clinically stable state by the cell fusion technique using polyethylene glycol [3]. It was hypothesized that the manifestation of measles virus in jejunum cells at certain times, probably at exacerbation, by an unknown mechanism results in the infection of nascent T-lymphocytes [9] and the modification of T-cell membranes. Then the modified T-lymphocytes can be recognized as foreign bodies and anti-modified T-lymphocyte sera will be produced as

auto-antibodies. These auto-antibodies, frequently found in the sera from MS patients in the exacerbation state, were proved to be detectable as lymphocytotoxic antibodies (10/12 patients, 83%) [10]. As the cell membrane of T-lymphocytes shares common antigens with human brain tissue [1, 2, 12], it is likely that the virus-induced lymphocytotoxic antibodies would further cross-react with brain cell membranes, leading to the immunopathological demyelination in the central nervous system of MS patients. We propose briefly that MS is an auto-immune disease triggered by a virus infection. The purpose of this paper is to confirm our hypothesis and to develop a new treatment for MS. Therefore, in order to inhibit the growth of measles virus in the patient's jejunum at exacerbation, we obtained an IgA-rich colostrum containing anti-measles neutralizing antibodies, which are resistant to proteases, and orally administered it to patients with MS.

Materials and methods

Preparation of anti-measles cow colostrum. Eight-month-pregnant Holstein cows were immunized subcutaneously with the Schwarz strain of measles virus (10^{5-6} TCID₅₀/ml x 5 ml) 5 times at 10-day intervals until calving.

Colostrum collected on the first 3 days (10 l, each) was immediately cooled and stored. Butter fat was removed by a cream separator and bacteria and red blood cells were removed by three centrifugations at 10 000 g for 30 min. Colostral non-fat milk was checked for bacteria (less than 30 000/ml) and *E. coli* before being approved for drinking by the Sendai Municipal Institute of Hygiene and then frozen in 100-ml units until administration.

Purification of immunoglobulin from cow colostrum. After casein had been removed by acid precipitation (pH 4.6), colostrum whey was applied to a DEAE cellulose column (DE₅₂, 1.8 x 28 cm) and eluted with 0.01 M phosphate buffer (pH 7.8). Each fraction was assayed for bovine IgG and bovine IgA by single radial immunodiffusion using anti-bovine serum IgG and anti-bovine serum IgA (Miles Laboratories, Inc., USA). After rechromatography of DE₅₂ the preparation containing IgG was applied to a Sephadex G-200 column (2 x 90 cm) and eluted with 0.1 M Tris-HCl buffer (pH 8.0) containing 0.2 M NaCl. After rechromatography of G-200, purified IgG was finally obtained. The preparation containing IgG was reapplied to a G-200 column. IgA fractions were then applied to a protein A CL4B affinity column and eluted with 0.1 M phosphate buffer (pH 8.0). After G-200 gel filtration, purified IgA was finally obtained. Both purified IgG and IgA showed a single precipitation band in agar diffusion.

Patients. Included in this study were 20 patients (5 males and 15 females) aged 9–40 years with definite MS as defined by the Schumacher Committee criteria [8]. The duration of the disease prior to entering the study varied from 1.0 to 9.5 years.

Clinical assessment. For a clinical assessment, disability scores adopted by the International Federation of Multiple Sclerosis Societies (IFMSS) and listed as the Minimal Record of Disability were used. The Minimal Record of Disability contains data with which comparisons can be made between one MS centre and another or between one

country and another. The Multiple Sclerosis Minimal Record of Disability consists of five sections: demographic information, functional systems, the disability status scale in MS, incapacity status and environmental status. For this report, the data for functional systems and the disability status scale were used. In evaluating functional systems, ratings are based on the results of standard neurological examinations (pyramidal, cerebellar, brain-stem, sensory, bowel and bladder, visual and mental functions). For the disability status scale, scoring is based on the functional systems rating on a 10-point scale.

Results

Characterization of anti-measles colostrum

Neutralizing antibody (NT) titres of cow colostrums to measles virus and HAI titre of cow serum are given in Table 1, where NT titres were measured using an Edmonston strain of measles and Vero cells. Also Table 2 shows the contents of IgA and IgG in colostrum non-fat milk. It is clear that 1st day colostrum contains the greatest amount of lactoglobulins with the highest NT titre when compared with 2nd and 3rd day colostrums. Market milk contains few lactoglobulins and no neutralizing antibodies to measles virus. Therefore, we studied the effect of the 1st day colostrum on altering the course of MS.

Table 1. Anti-measles NT titre of colostrum and HAI titre of serum

Cow no.	Year	Milking (day)	NT titre	HAI
253	1981	1st	<8	<8
		2nd	<8	
		3rd	<8	
	1982	1	32	8
		2	16	
		3	8	
	1983	1	6400	64
		2	6400	
		3	6400	
200	1980	1st	<8	<8
		2nd	<8	
		3rd	<8	
	1981	1	64	8
		2	32	
		3	32	
	1982	1	4096	256
		2	2048	
		3	512	
	1983	1	3200	128
		2	3200	
		3	1600	

Table 2. Contents of immunoglobulins in cow colostrum (mean of 12 colostrums, mean \pm SD)

Milking (day)	IgA (mg/100 ml)	IgG (mg/100 ml)	IgM (mg/100 ml)
1st	292 \pm 15	3023 \pm 121	167 \pm 12
2nd	125 \pm 11	1574 \pm 67	47 \pm 7
3rd	55 \pm 5	631 \pm 32	14 \pm 3

Table 3. NT titre of purified IgG and IgA to measles virus

	Colostrum	Purified IgG	Purified IgA
Ig concentration (mg/ml)	67	5.4	1.5
NT titre to measles	16	8	16
Specific activity (/mg Ig)	0.24	1.5	10.7

Next, in order to characterize an effective Ig component having anti-measles neutralizing antibodies in colostrum, IgG and IgA were purified as described in Materials and methods. As shown in Table 3, the neutralizing activity of IgG and IgA against measles virus (Edmonston strain) was 1:1.5/mg and 1:10.7/mg respectively. Therefore, it seems that the neutralizing activity of IgG in colostrum is quantitatively dependent (Table 2) and that IgA is important qualitatively for inhibiting measles virus growth in the jejunum.

As for the stability of colostrum's neutralizing activity against proteases, we reported in a previous paper that treatment with trypsin (100–800 μ g/ml) for 1 h did not affect the neutralizing titres [4].

Clinical effect of anti-measles colostrum on MS

Three kinds of cow colostrums were used; a high NT titre to measles virus (512-5120), a low NT titre to measles virus (8-32), and a negative NT titre to measles virus (< 8). A total of 20 MS patients were orally given 100 ml of IgA-rich colostrum every morning for 30 days. For each patient, the change in disability scores before and after the oral administration of colostrum was compared. As shown in Table 4, of 7 high NT titre anti-measles colostrum recipients 5 patients improved and 2 remained unchanged. As summarized in Table 5, anti-measles colostrum significantly improved the condition of MS patients ($P < 0.05$).

Table 4. Clinical assessment by disability scores in MS patients treated with anti-measles colostrum

Case Age Sex	Disability scores		After ad- ministration	Clinical assessment
	Before colostrum ad- ministration			
OM 32 Female	Functional systems	(6)	(3)	Improved
	pyramidal function	2	2	
	cerebellar function	2	1	
	sensory function	1	0	
	bladder function	1	0	
Disability status scale	(2)	(1)		
SY 14 Female	Functional systems	(11)	(5)	Improved
	pyramidal function	2	0	
	brain-stem function	2	0	
	sensory function	2	0	
	visual function	5	5	
Disability status scale	(2)	(1)		
TY 23 Male	Functional systems	(5)	(3)	Improved
	pyramidal function	1	1	
	cerebellar function	2	0	
	brain-stem function	2	2	
Disability status scale	(2)	(1)		
IT 28 Female	Functional systems	(8)	(8)	Unchanged
	pyramidal function	2	2	
	brain-stem function	2	2	
	sensory function	3	3	
	bladder function	1	1	
Disability status scale	(3)	(3)		
WY 33 Female	Functional systems	(8)	(8)	Unchanged
	pyramidal function	3	3	
	sensory function	3	3	
	bladder function	2	2	
Disability status scale	(3)	(3)		
TM 45 Female	Functional systems	(16)	(15)	Improved
	pyramidal function	5	5	
	sensory function	4	4	
	bladder function	3	2	
	visual function	4	4	
Disability status scale	(7)	(7)		
YO 32 Male	Functional systems	(10)	(8)	Improved
	pyramidal function	2	2	
	cerebellar function	3	3	
	brain-stem function	2	0	
	sensory function	1	1	
mental function	2	2		
Disability status scale	(3)	(3)		

Table 5. Effects of anti-measles colostrum on MS patients

Colostrum	High NT titre to measles virus (512–5120)	Low NT titre to measles virus (8–32)	Negative NT titre to measles virus (< 8)
Total recipients	7	8	5
Improved cases	5*	5*	0
Unchanged cases	2	3	2
Worsened cases	0	0	3

*($P < 0.05$)

Discussion

The aetiology of MS is still unknown and there is no specific treatment. Steroid hormones have been commonly used for exacerbations but have severe side-effects. Jacobs et al. [5] reported that intrathecal interferon reduced exacerbations of MS, but serial lumbar injections of interferon are painful. Mertin et al. [6] reported on the marginally beneficial effect of immunosuppressives (azathiopurine, antilymphocyte globulin and prednisolone), which were especially effective for HLA-A3-positive patients. However, immunosuppressive treatments also cause various side-effects. On the other hand, our oral colostrum administration did not cause any side-effects. Present data show that colostrum non-fat milk containing lactoglobulin with NT titre to measles virus seems to hinder the clinical course of MS. Recently, Watanabe et al. [11] reported that infecting rats with murine coronavirus JHM led to the development of demyelinating encephalomyelitis several weeks to months post infection. Lymphocytes from these diseased Lewis rats were restimulated with basic myelin protein and adoptive transfer of these cells led to lesions resembling those of experimental allergic encephalomyelitis in recipients. This model demonstrates that a virus infection in CNS tissue is capable of initiating an auto-immune response, which may be of pathogenic importance.

We would like to propose that MS is also an auto-immune disease triggered by a morbillivirus infection in the small intestine.

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