Detection of Elevated Levels of 2-5A Synthetase in Serum from Children with Various Infectious Diseases

HIROTOSHI SUGINO,^{1*} IKUKO MITANI,¹ MICHIO KOIKE,¹ TEISUKE KODAMA,¹ JUNKO SOKAWA,²
HIROAKI SAWAI,³ KAICHIRO ISHIBASHI,⁴ MASAO ITOH,⁴ SHOICHI WATANABE,⁴ AND
YOSHIHIRO SOKAWA⁵

Department of Pediatrics, Wakayama Medical College, Wakayama 640,¹ Radioisotope Research Center, Kansai Medical University, Moriguchi-shi, Osaka 570,² Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113,³ Eiken Immunochemical Laboratory, Ooji, Kita-ku, Tokyo 114,⁴ and Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606,⁵ Japan

Received 13 February 1986/Accepted 23 May 1986

By a sensitive radioimmunoassay method, (2'-5')oligoadenylate synthetase was detected in serum from patients with viral, bacterial, or mycoplasmal infections at elevated levels compared with enzyme levels in serum from healthy individuals and patients suffering from noninfectious diseases.

(2'-5')Oligoadenylate synthetase (2-5A synthetase), which catalyzes the formation of 2-5A from ATP in the presence of double-stranded RNA, is an enzyme induced in the cell by the addition of interferons (5). The 2-5A system, which is composed of 2-5A synthetase, 2'-5'-phosphodiesterase and 2-5A-dependent RNase, is thought to play an important role in the antiviral function of interferon (7). The induction of 2-5A synthetase was also shown in tissues of animals infected with viruses (6, 16). Thus, the increase in 2-5A synthetase is one of the most sensitive biochemical markers of cells in response to interferon or viral infections. In fact, high levels of the synthetase activity were detected in peripheral lymphocytes from patients injected with interferon (8, 13) and from patients suffering from various virus infections (1, 2, 3, 14, 17). Recently, the assay of 2-5A synthetase was suggested to be a useful prognostic indicator for acquired immunodeficiency syndrome (9, 10). However, the isolation of lymphocytes from peripheral blood is so complicated that in daily clinical work this assay method has not been used widely. If we could detect 2-5A synthetase activity in serum instead of in lymphocytes, the application of this enzyme assay in diagnosis would become very convenient. Therefore, we tried to detect 2-5A synthetase activity in serum obtained from children suffering from several infectious diseases by a sensitive radioimmunoassay method.

In this study, we examined 63 serum samples from healthy adult individuals and 101 samples from the following 70 children: 17 patients with viral diseases, including 7 with respiratory syncytial virus infection, 6 with rotavirus infection, 2 with measles, and 1 each with varicella and herpangina; 13 patients with bacterial infections, including 5 with scarlet fever, 2 with pertussis, 2 with appendicitis, and 1 each with bacterial meningitis, purulent lymphadenitis, omphalitis, and furuncle; 11 patients with mycoplasmal pneumonia; 23 patients suffering from noninfectious diseases, including 12 with microhematuria, 2 with arrhythmia, and 1 each with hypogranulocytosis, hypothyroidism, nephropathy, transient chest pain, neonatal jaundice, congenital wheezing, intracranial bleeding, idiopathic thrombocytopenic purpura, and atopic dermatitis; and 6 cases of health consultation. The diagnoses of both respiratory

syncytial virus and mycoplasmal infections were confirmed serologically; rotavirus infection was diagnosed by the detection of virus in the stool with the Rotarex kit (Daiichi Pure Chemical Co., Tokyo, Japan). Scarlet fever was diagnosed by typical rash, fever, tonsillitis, and the presence of *Streptococcus pyogenes* in throat culture. The acute phase of viral and mycoplasmal infection was considered to be within 7 days after onset of the illness. All serum samples were kept frozen at -40°C until assayed.

The levels of 2-5A synthetase activity were measured by the modified method of Shimizu and Sokawa (15) and Sawai et al. (11, 12). The assay kit was constructed in Eiken Immunochemical Laboratory (Tokyo, Japan). The kit contains nonlabeled ATP as a substrate; poly(I)-poly(C) agarose gel, which adsorbs and activates 2-5A synthetase; [¹²⁵I]-2-5A; and a suspension of rabbit anti-2-5A and a second antibody, goat anti-rabbit antibody, for radioimmunoassay for 2-5A product. A 50-μl amount of serum was used for an assay. The minimal sensitivity of this assay is 5 fmol per tube, and the upper limit is 405 fmol per tube. The reduction of serum concentration made a straight line through the original point (data not shown). The 2-5A synthetase activity was expressed as pmol/h per dl of serum.

When we examined 2-5A synthetase activity in serum from each of the 63 healthy adult volunteers, appreciable amounts of this enzyme could be detected and the mean level and standard deviation was calculated to be 40 ± 41 pmol/h per dl of serum (Fig. 1). In 31 serum samples from 29 children with noninfectious disease, the synthetase level and standard deviation was 70 ± 42 pmol, which was slightly higher than that in serum from adult individuals ($P < 0.001$). On the other hand, the mean level and standard deviation of 2-5A synthetase activity in 21 serum samples from 15 patients in the acute phase of viral diseases was 456 ± 250 pmol/h per dl of serum, and the range was from 86 to >810 pmol (Fig. 1). In patients suffering from respiratory syncytial virus or rotavirus infections, the levels of serum 2-5A synthetase were highest on day 3 or 4 after onset of the illness, followed by a decrease during the convalescent phase of disease (Table 1). The titers of complement fixation antibody against respiratory syncytial virus were elevated when 2-5A synthetase levels declined (Table 1). These results clearly indicate that the levels of serum 2-5A synthetase from patients in the acute phase of viral infections were

* Corresponding author.

FIG. 1. Levels of 2-5A synthetase in the serum. Circles and ovals represent one and five persons, respectively. The bars show mean value $\pm$ standard deviation.

enhanced significantly, in contrast with those of serum from healthy adults or patients with noninfectious disease ($P < 0.001$).

All 10 serum samples from nine patients with mycoplasmal infections which were diagnosed as primary atypical pneumonia showed increased levels of 2-5A synthetase in their acute phase (308 ± 118 pmol) (Fig. 1). However, the enzyme activities in serum decreased during recovery from the disease, when the increased complement fixation antibody titer was observed (Table 2). In 13 patients with bacterial infections, the enzyme level in serum was 112 ± 88 pmol, which was not as high as that in patients with viral infections ($P < 0.001$), except for one patient suffering from scarlet fever (344 pmol) (Fig. 1). Mycoplasma, some bacteria, and

bacterial endotoxin are known to be interferon inducers (18), but the results described above indicate that they are not as good an inducer of 2-5A synthetase as viruses are. It was also shown that the injection of interferon made the level of serum 2-5A synthetase increase (data not shown).

It has been reported that the activity of 2-5A synthetase was significantly increased in peripheral blood mononuclear leukocytes from patients with several idiopathic inflammatory diseases, such as systemic lupus erythematosus, sarcoidosis, and Vogt-Koyanagi-Harada disease (4). However, there was no relationship between the levels of 2-5A synthetase and those of C-reactive protein, which is an indicator of inflammatory diseases (Fig. 2). Even in some bacterial infections in which high levels of C-reactive protein were

TABLE 1. 2-5A synthetase activity in serum during the course of respiratory syncytial virus and rotavirus infections

Day(s) after onset of clinical symptoms	2-5A synthetase activity (complement fixation antibody titer) in serum from the following patients ^a infected with:												
	Respiratory syncytial virus							Rotavirus ^b					
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	341	174 (<4)											492
2									>810				
3	>810 (<4)		628 (<4)										
4		479 (8)	>810 (<4)			339 (<4)	553 (16)						
5				572 (<4)	(<4)			129		258	708		
6			700 (<4)										
7									224				292
8		261 (16)	110 (<4)				23 (128)	116			592		
9												198	
10	47 (32)		(4)	242 (32)									
11					144 (16)	137 (16)							
13			(8)										
14											232		

^a Patients are identified by number.

^b Only 2-5A synthetase activity is given for this group.

TABLE 2. 2-5A synthetase activity in serum during the course of mycoplasmal infection

Days after onset of clinical symptoms	2-5A synthetase activity (complement fixation antibody titer) in serum from the following patients:										
	14	15	16	17	18	19	20	21	22	23	24
2										266 (<4)	
3				(<4)		145 (4)					
4									230 (<4)		
5			268 (<4)				151 (<4)				
6	>810 (<4)	236 (32)	332 (<4)								
7					322 (<4)						324 (<4)
8				>810 (16)				159 (32)			
9										48 (16)	
10			306 (<4)			44 (16)		138 (32)	171 (16)		
11		97 (256)									
13			552 (4)					72 (128)			240 (4)
14					74 (16)						
15	(32)						72 (32)				
20			50 (32)								
21					35 (32)						

observed, the activities of 2-5A synthetase remained at low levels. Thus, the elevation of 2-5A synthetase levels in serum reported here was not the result of a nonspecific inflammatory response.

Although the reason why 2-5A synthetase appears in serum after viral infection or interferon injection is not clear, it may be due to the degeneration of lymphocytes during their life cycle, since this enzyme may not be a secreted one.

As shown here, for the measurement of 2-5A synthetase in

clinical use, serum could be substituted for lymphocytes; serum is very convenient for this purpose. For example, lymphocytes should be isolated immediately after venipuncture by density gradient centrifugation using at least 2 ml of blood, whereas the isolation of serum is a simple procedure and the volume of serum needed for an assay is as small as 50 μ l.

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FIG. 2. Correlation between 2-5A synthetase and C-reactive protein in serum in the acute phase of viral, mycoplasmal, and bacterial infections.

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