One can expect little, if any, influence of iron reduction on SR inducible by IFN, and it has been borne out by the actual results [23].

2. Interferon (IFN)

The SR rate to IFN increases in parallel with the duration of therapy, extending from six through 12 to 18 months [24]. Although longer treatment times with IFN increases SR, the patients who respond to long-term IFN are restricted to those who clear HCV RNA from serum while they are on IFN.

It has to be pointed out that there are patients who fail to archieve SR, but in whom ALT levels continue to be normalized despite persistent high titers of HCV RNA during IFN. ALT levels in these patients elevate after the withdrawal of IFN. As long as they are continued on IFN, however, ALT levels stay normalized, accompanied by improved histopathology in the liver. Long-term use of low-dose IFN, for suppressing necroinflammatory processes in the liver, is proposed by Shiffmann et al. [25].

Kasahara et al. [26] and Okanoue et al. [27] have reported a decreased incidence of HCC in transient responders to IFN, in whom ALT normalized and HCV RNA was cleared only while they were on IFN, in long-term follow-ups after the completion of IFN therapy. The effect of IFN in this situation is produced by its ability to retard the development of HCC by a time span of three to five years [26]; transient responders develop HCC at a rate comparable to that of nonresponders beyond that time point. Based on these observations, it may be possible to postpone the development of HCC in transient responders by placing them on IFN regularly at intervals of a few years.

3. Glycyrrhizin

Glycyrrhizin is the active substance in extracts of the root of *Glycyrrhiza glabra* (licorice root). Glycyrrhizin is composed of one molecule of glycyrrhetinic acid coupled with two molecules of glucuronic acid. It has been used for ages as an anti-allergic drug in China, and as a substitute for sucrose and an anti-ulcer drug in Europe [28]. In Japan, glycyrrhizin is supplied as an intravenous drug for the past 60 years, principally for treatment of urticaria and dermatitis. The indication for glycyrrhizin has been extended to chronic hepatitis, on the premise that hepatitis would be induced by allergic reactions.

Encouraged by the early success of glycyrrhizin in reducing ALT levels in sporadic cases of chronic hepatitis, Suzuki et al. conducted a randomized double-blind trial of glycyrrhizin in 1977 in patients with chronic hepatitis, most of whom turned out to be infected with HCV. The results were introduced by them to Western societies of medicine in 1983 [29]. They showed that glycyrrhizin can lower ALT levels significantly. Since then, glycyrrhizin has been used for symptomatic treatment of patients with chronic hepatitis in Japan. Hino et al. [30] went on to demonstrate that glycyrrhizin not only lowers ALT levels, but also suppresses inflammation in the liver. As mentioned above, Arase et al. [7] have shown that long-term use of glycyrrhizin for more than 15 years can significantly decrease the incidence of HCC in patients with chronic hepatitis C.

Although glycyrrhizin evidently suppresses necroinflammatory processes in the liver, the mechanism is not certain despite many studies focusing on it. Glycyrrhizin in large doses can induce pseudoaldosteronism in a few patients, which is easily controlled by regular tests for serum electrolytes and prescribing spinorolactone to the patients with critically low K^+ levels. The reproducible and reliable activity of glycyrrhizin to reduce ALT levels with minimal side effects has resulted in many clinical trials with it in patients with chronic hepatitis C in a number of European and Asian countries.

4. Ursodeoxycholic acid (UDCA)

UDCA represents one of the hydrophilic bile acids, originally identified in the bile of bears. It is contained in human bile in small amounts as a secondary bile acid. UDCA has been used for a long time in China as a drug for bowel disturbances and for stimulating secretion of bile.

In 1976, Yamanaka et al. [31] conducted a randomized controlled trial in patients with chronic hepatitis using placebo, 150 and 600 mg/day of UDCA, and found that 600 mg/day UDCA significantly decreased the levels of ALT, aspartate aminotransferase and γ-glutamyl transpepitidase. Their results were corroborated by Buzzelli et al. in 1991 [32].

Trauner et al. [33] reviewed in detail the effect of UDCA on chronic hepatitis; it has not been fully explained, as yet. The present understanding of the pharmaceutical effects of UDCA on chronic hepatitis includes: (1) replacement of endogenous toxic bile acids; (2) cytoprotective effects on hepatocytes and bile duct cells; and (3) immonomodulatory activity. UDCA stimulates the alimentary tract as a side effect, an effect which is circumvented by timing the intake to just before each meal.

5. Ribavirin

Ribavirin is an antiviral drug that has been in use for many years. By virtue of its ability to enhance the capacity of IFN to eradicate HCV, combined use of IFN and ribavirin has become the first therapeutic choice in the treatment of chronic hepatitis C. Ribavirin by itself, however, has a high activity to suppress ALT levels, although it does not prohibit the replication of HCV. Monotherapy with ribavirin was started by Richard et al. [34] in 1991, and followed by several groups of investigators thereafter. On the grounds that ribavirin cannot clear HCV from serum, even though it suppresses ALT, ribavirin monotherapy has been proposed to be ineffective for treatment of chronic hepatitis C [35]. Ribavirin has side effects represented by hemolytic anemia, and cholelithiasis is reported in recipients who have received long-term treatment. Due to the capacity of ribavirin for suppressing serum ALT, it is hard to dismiss ribavirin monotherapy from the list of symptomatic therapies for chronic hepatitis C.

6. Corticosteroids (CS)

Persistent HCV infection elicits a variety of autoantibodies [36], typified by antinuclear antibodies, and accompanies many extrahepatic diseases such as cryoglobulinemia, in which HCV proteins are involved, along with IgM rheumatoid factors [37]. Although

CS have not been evaluated for long-term efficacy in symptomatic therapy of chronic hepatitis C, Fong et al. [38] and Thiele et al. [39] refer to the effect of CS on patients with chronic hepatitis C. Their results agree on the capacity of CS for lowering serum ALT levels; the reduction is accompanied by increased HCV RNA titers in serum, however. CS are expected to lower ALT levels by means of a cytoprotective activity or immunosuppressive capacity, or both. Hence, CS would be particularly effective in patients with chronic hepatitis C who have stigmata of autoimmune hepatitis represented by high-titered autoantibodies.

7. Cyclosporine A (CsA)

CsA suppresses cell-mediated immune responses and, therefore, has been widely used for preventing graft-versus-host disease and allograft rejection in recipients of transplants, as well as for treatment of autoimmune diseases. Although the exact mechanism of hepatocyte injury in chronic hepatitis C is not clear, as yet, the involvement of cellular immunity mediated by lymphocytes is strongly suspected.

Kakumu et al. [40] placed ten patients with chronic hepatitis C on daily 1.5-4.0 mg/kg of CsA. They found a significant decrease in ALT levels while the patients were given CsA, with minimal side effects inclusive of renal injuries.

8. Interleukin-10 (IL-10)

Frequent suppression or normalization of serum ALT levels has been reported by McHuchison et al. [41], who gave 4 or 8 μg/kg/day of IL-10 to 16 patients with chronic hepatitis C for 28 days. This was also verified by Nelson et al. [42], who prescribed the same doses of IL-10 to 24 patients for 90 days. Significant changes in HCV RNA were not seen during IL-10 in either trial, however. ALT returned to pretreatment levels after withdrawal of IL-10. Nelson et al. [42] compared paired biopsy specimens, taken before and after IL-10 therapy, and recognized significant suppression of fibrosis in the liver following treatment. All in all, IL-10 may be another promising candidate for symptomatic therapy of chronic hepatitis C.

Application of symptomatic therapies to patients with chronic hepatitis C

Optimal doses are not determined for any drugs for the symptomatic therapies of chronic hepatitis C reviewed above. In terms of practical care for patients with chronic hepatitis C, therefore, many issues remain unsolved, including long-term side effects of these drugs.

We have gained experience on symptomatic therapies during the past 15 years, starting when chronic hepatitis C was called non-A, non-B hepatitis. Optimal therapeutic doses have been worked out for some of these drugs and will be presented with some examples.

Of our series of 300 patients with chronic hepatitis C, with or without compensated LC, who have received symptomatic therapy for 10 years or longer, HCC developed in

only six (2%). The outcome is biased in that only the patients who received symptomatic therapies for a long while have been selected and followed, without appropriate controls. Even with such reservations, the incidence of HCC in them (2% in 10 years) would be very low by any standards and in comparison with historical controls.

1. UDCA

To the patients with serum ALT levels less than 60-70 IU/L, 600 mg of UDCA daily in three divided doses is given with the goal of maintaining ALT below 40 IU/L. As shown in Table 1, ALT levels decrease by one rank or more by this therapeutic regimen. When ALT decreases to around 30 IU/L, the dose of UDCA is reduced to 300 mg. An example is shown in Figure 1 for a case in whom ALT levels were controlled for three years using UDCA.

Table 1. Average ALT levels before and during the administration of daily 600 mg ursodeoxycholic acid (UDCA) in 52 patients with chronic hepatitis C

Mean ALT levels during 6 months or longer (IU/L)	Pretreatment	During UDCA[a]
>100	6 (12%)	6 (12%)
70-100	22 (42%)	9 (17%)
50-70	17 (33%)	12 (23%)
32-50	7 (13%)	17 (33%)
<32	0	8 (15%)

[a] Lower than pretreatment levels ($p < 0.05$).

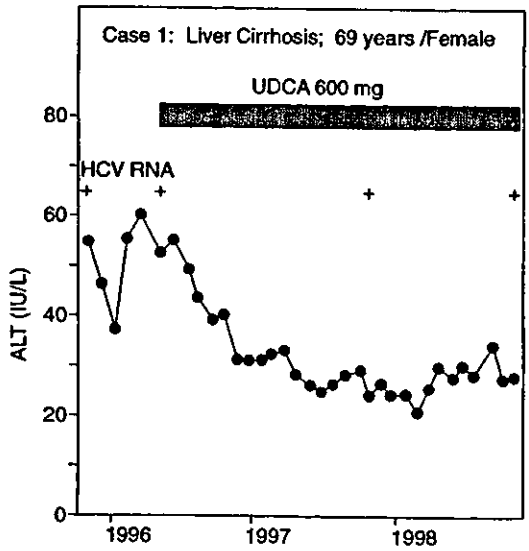

Figure 1. Symptomatic therapy with ursodeoxycholic acid alone.

2. Glycyrrhizin

For patients with serum ALT levels higher than 50-60 IU/L, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical Company, Tokyo Japan) is used. One ampoule of SNMC contains 40 mg of glycyrrhizin in a 20-ml solution, supplemented with 20 mg/ml glycine and 1 mg/ml cysteine. With constraints for being an intravenously administered drug, SNMC does reduce ALT levels and has been used in tens of thousand patients with chronic hepatitis for more than 20 years in Japan, with minimal side effects. Patients are started on two ampoules, or 40 ml, of SNMC per day (equivalent to 80 mg of glycyrrhizin), and if they fail to respond by lowering ALT below 60 IU/L, the dose can be increased to five ampoules, or 100 ml, of SNMC per day (equivalent to 200 mg of glycyrrhizin). The dose of SNMC is gradually reduced, once a low ALT level is archieved, to the maintenance dose of two or four ampoules per day (equivalent to 80 or 160 mg of glycyrrhizin) (Figure 2).

When SNMC alone cannot suppress serum ALT below 50 IU/L, or if a decreased dose of SNMC is desired, UDCA may be combined for controlling ALT. Conversely, when UDCA alone cannot reduce serum ALT below 50 IU/L, SNMC may be added for suppressing ALT levels below 50 IU/L (Figure 3).

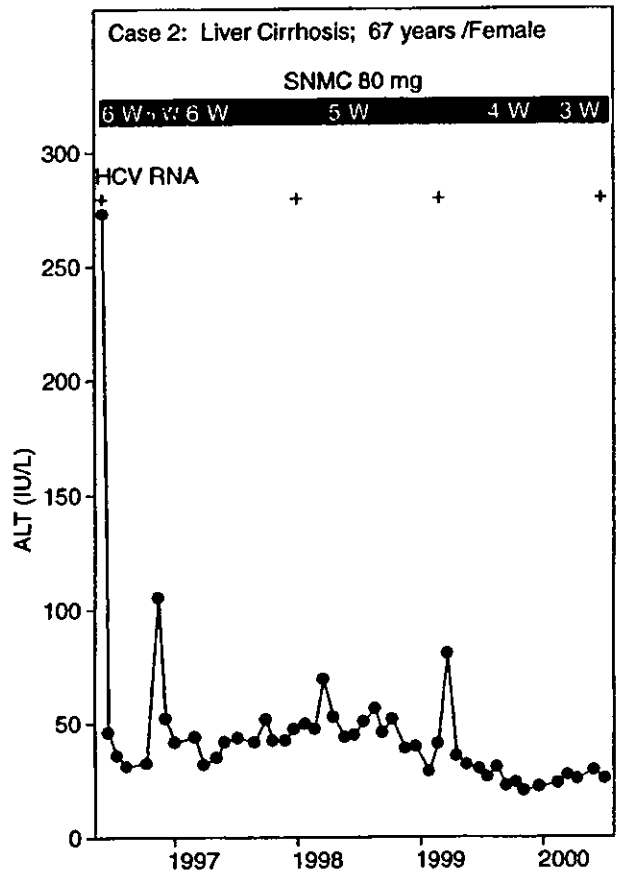

Figure 2. Symptomatic therapy with varying doses of Stronger Neo-Minophagen C.

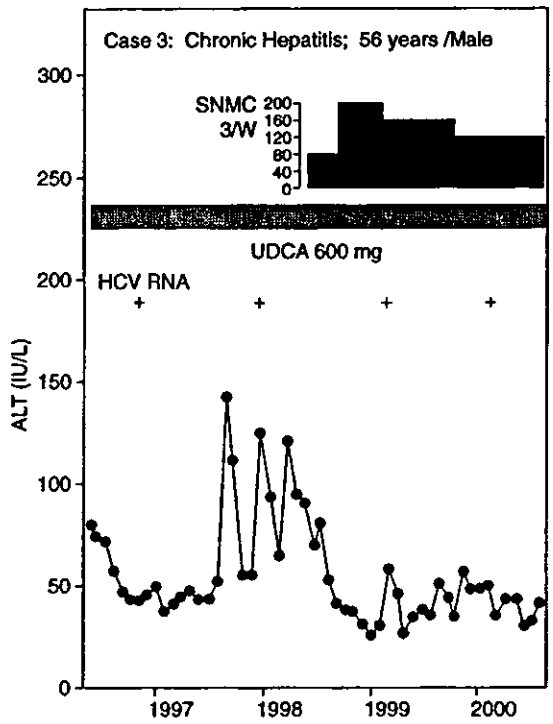

Figure 3. Treatment with Stronger Neo-Minophagen C superimposed on ursodeoxycholic acid.

3. Phlebotomy

Biweekly phlebotomy of 400 ml is repeated until serum ferritin decreases to 10-20 ng/ml. Usually, the target level of ferritin is reached within five to seven cycles of phlebotomy. If and when ALT increases thereafter, phlebotomy of 400 ml is performed for controlling ferritin levels. In most cases, a phlebotomy every four to six months suffices to maintain low ALT levels.

One patient received phlebotomy when SNMC and UDCA could not suppress ALT levels below a desired level (Figure 4). Phlebotomy was effective in controlling ALT levels.

4. IFN

In many clinical trials reported in the literature, IFN at a dose of 3 MU three times a week normalizes ALT in 50-60% of the recipients. Sporadic cases are observed which achieve continued normal ALT levels, even though they keep serum HCV RNA (albeit in somewhat lowered titers) and fail to clear it from serum. A case is presented in Figure 5 in which SNMC and UDCA failed to sufficiently suppress ALT levels. Additional IFN-α at a dose of 3 MU three times a week normalized ALT, whereupon SNMC and UDCA were withdrawn. IFN-α was curtailed to two times a week; ALT was maintained for longer than three years.

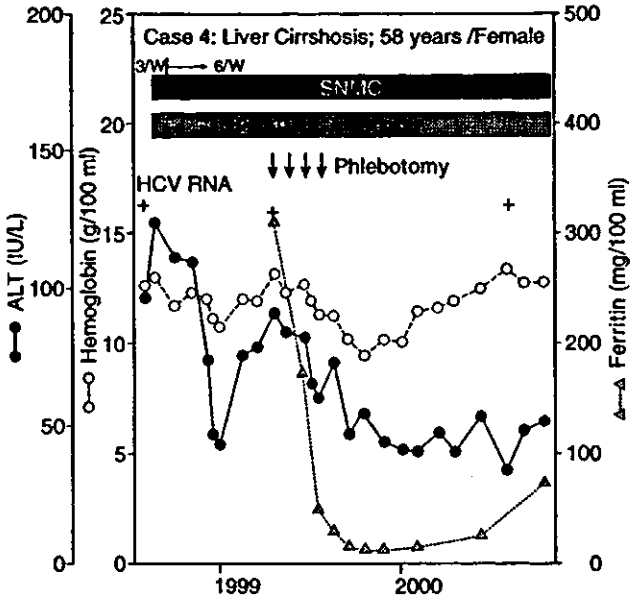

Figure 4. Triple treatments with Stronger Neo-Minophagen C, ursodeoxycholic acid and occasional phlebotomies.

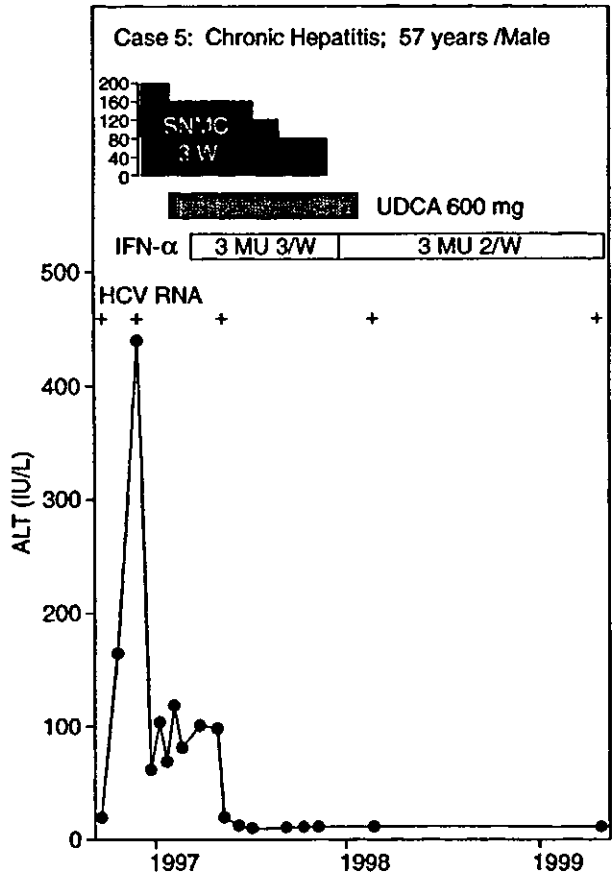

Figure 5. Induction with Stronger Neo-Minophagen C, followed by urosdeoxycholic acid and maintenance with low-dose interferon-α.

5. CS

CS at a dose of 5-10 mg/day can keep serum ALT levels low in some patients with chronic hepatitis C. Most of these patients have antinuclear antibodies in high titers in serum. An example is shown in Figure 6 for a case with antinuclear antibody in a titer of 1:1,280 before the patient was placed on IFN. It went up to 1:260 during IFN treatment, accompanied by increased ALT levels. On the withdrawal of IFN, ALT decreased, but HCV RNA turned positive. For control of ALT levels, 5 mg/day CS was given to the patient, which was effective in lowering ALT to within the normal range.

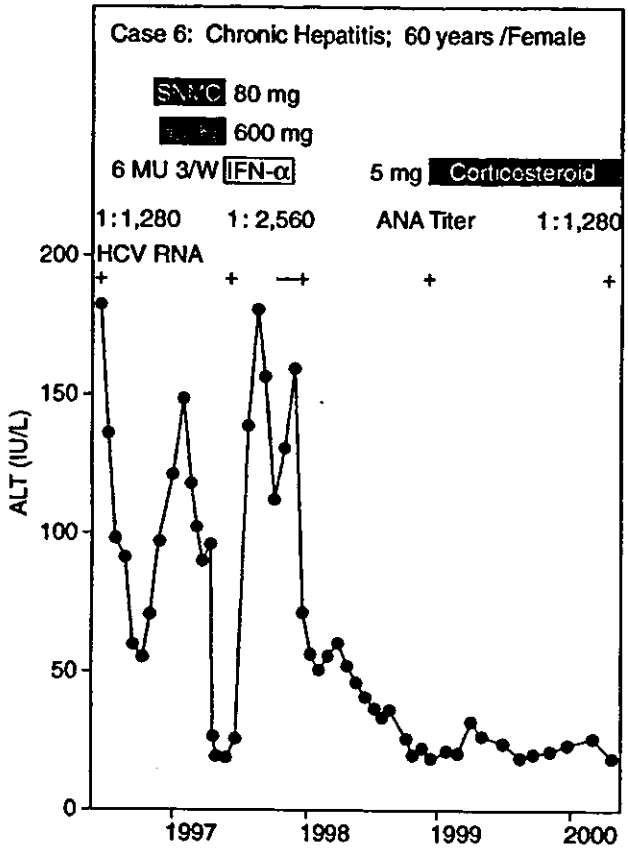

Figure 6. Normal ALT levels attempted by a triple therapy with Stronger Neo-Minophagen C, ursodeoxycholic acid and interferon-α. Levels were successfully maintained by a low-dose corticosteroid in a female patient with chronic hepatitis C who was seropositive for antinuclear antibodies.

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Prevention of and Measures against Needlestick Accidents

JMAJ 46(4): 156–160, 2003

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Abstract: Needlestick accident is a term that symbolically expresses blood-related infections seen among healthcare workers. Healthcare workers must understand the nature of such infections, and learn how to prevent and deal with needlestick accidents if they are to protect themselves from such infections. The background and specific preventive measures against HBV, HCV, and HIV infections are herein provided, along with daily measures to be taken so that, more importantly, healthcare workers will become aware that all blood and anything possibly contaminated by blood are potential source of infection.

Key words: HBV infection; HCV infection; HIV infection; Needlestick accidents

Introduction

Needlestick accident is a term that is used to represent injury that is incurred by the handling of instruments with a patient's blood.

Although such injuries are always likely to occur during daily medical practice, this is the type of accident that can certainly be reduced if each healthcare worker is careful with himself/herself and others.

While HBV (hepatitis B virus) initially drew attention as the cause of infections caused by such injuries, followed by HIV (human immunodeficiency virus) and HCV (hepatitis C virus), it must be recognize that, in reality, infection can be caused not only by these viruses but by any pathogen in a patient's blood. Although patients with HBV, HCV, and HIV infections tend to get the greatest attention, and the attempts to identify only such patients are prevalent, not all patients can be actually identified since there are patients who have not been tested for these three diseases and unknown carrier of these diseases. The most important thing, therefore, is to consider all blood as a source of infection.

General preventive measures and measures used to deal with accidents caused by the three aforementioned infections are herein discussed.

General Measures for Prevention of Accidents

General measures for prevention of accidents

This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol. 127, No. 3, 2002, pages 367–370).

have been widely taught in the past 20 years, since guidelines for measures against nosocomial infections, such as HBV infection that became a problem first, were prepared and publicized by the Hepatitis B Research Team of the Hepatitis Research and Liaison Council Division of the Health and Welfare Ministry at the time in 1982.[1] The guidelines also became the bases for guidelines for HIV infection and HCV infection.

They can be summarized as follows:

- Healthcare workers should prepare for invasive medical practice by protecting themselves with gloves, protective gown, and protective glasses.
- Fingers should not touch the tip of an edged instrument during surgery to the extent possible.
- Edged instruments should be used with the utmost care.
- Edged instruments should not be handled carelessly.
- Instruments that have been used should be immediately rinsed with water or soaked in antiseptic solution or water.
- Disposable needles and edged instruments should be disposed into a special container in accordance with specified methods.

However, even if these acts were carried out very carefully, it is difficult to avoid accidents of irresistible force during medical practice. Be that as it may, as a specialist in the field of viral hepatitis, on an average, I still annually encounter at least one case of hepatitis C that have occurred within 2 to 3 months of receiving medical care. This is solid evidence that healthcare workers mediate HCV infection from patient to patient, and shows that the basic daily preventive measures mentioned earlier have still not been thoroughly implemented. The fact that such situations still remain suggests the necessity for healthcare workers to be aware that the risks are rising.

It is necessary for hospitals to make an effort to maintain awareness of infections among healthcare workers by running workshops from time to time through something like a committee especially set up for prevention of nosocomial infections.

At medical institutions, people are commonly seen eating or drinking with their preventive gowns still on. The first step in preventing nosocomial infections may be to correct the daily habits of healthcare workers by encouraging them to wash their hands, wash their faces, and take off their preventive gowns after work and before eating or drinking.

Response to Needlestick Accidents

There is a need to check the presence of new HBV or HCV infection during regular medical checkups, and to see the changes in HBs antibody titer for HBV infection. While the aforementioned guidelines previously stated that, depending on the department, tests should be conducted every two to three months for HBV, once a year seems sufficient now that the incidence of actual infections has decreased thanks to various responses.

Next, as something common to all needlestick accidents, wounds should be washed under running water and blood should be squeezed out as soon as it is discovered. The wound must also be disinfected with a disinfectant. At the same time, it should be verified whether or not the patient has been infected with HBV, HCV, or HIV, and the following measures should be taken once it is confirmed that the patient was infected. In this case, it is important to contact the person in charge of a task force for nosocomial infections, and to record the details.

1. Prevention of HBV infection

At least 15 years have past since HB vaccine came onto the market, and all healthcare workers under the age of 31 have supposedly received HB vaccine.

If they had received HB vaccine before or when they started working as healthcare workers, most of such people would have been in their 20s. Therefore, since the HBs antibody

Table 1 Changes in the Ratio of Positive HBs Antibody Test and the Geometric Mean Antibody Titer Following rHB Vaccination in Different Age Groups[2]

Age group (years)	Item	Months after vaccination			
		Before inoculation	1 month later	6 months later	7 months later
>10	Positive ratio	0/144*	49/144 (34.0)	137/142 (96.5)	130/132 (98.5)
	Antibody titer	<0.6**	4.0	60.1	420.7
10–19	Positive ratio	0/197	42/197 (21.3)	192/197 (97.5)	193/194 (99.5)
	Antibody titer	<0.6	5.1	33.9	363.1
20–29	Positive ratio	0/703	113/703 (16.1)	614/696 (88.2)	651/668 (97.5)
	Antibody titer	<0.6	2.6	19.5	158.5
30–39	Positive ratio	0/406	38/406 (9.4)	308/401 (76.8)	368/386 (95.3)
	Antibody titer	<0.6	2.5	13.5	64.6
40≤	Positive ratio	0/281	28/281 (9.9)	194/279 (69.5)	261/273 (95.6)
	Antibody titer	<0.6	2.3	9.1	36.3
Total	Positi ve ratio	0/1,731	270/1,731 (15.6)	1,445/1,715 (84.3)	1,603/1,653 (97.0)
	Antibody titer	<0.6	3.1	19.6	117.3

* Number of positive cases/Number of cases from whom blood was collected
** Geometric mean antibody titer: mIU/ml ( ): Percentage of positive antibody

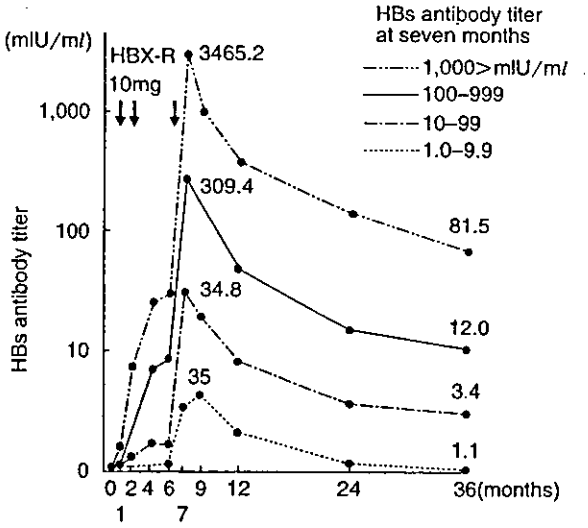

Fig. 1 Changes in the antibody titer based on HBs titer seven months following the initial inoculation[3]

acquisition rate has been at least 98% in all vaccine-related trials, and cases without positive HBs antibody include cases with positive HBc antibody, which show that a person has been infected with HBV, approximately 100% of them are thought to have acquired HBs antibody. In particular, since recombinant vaccines have started to be used, the rate of positive HBs antibody continues to improve even with age,[2] as shown in Table 1, and it is speculated that HBs antibody emerges at least once in vaccinated individuals.

However, since HBs antibody that has been acquired through vaccination decreases with time, as shown in Fig. 1, it may be necessary to get additional vaccinations.[3] On the other hand, however, some have recently reported

that there is no such need, based on the fact that results of a long-term follow-up study on people who received vaccinations during early childhood show that they did not get infected even without additional inoculation when HBs antibody had turned negative. However, the data in these reports may not be credible as to whether or not infection was truly prevented if circumstance made it difficult for infection to occur, such as through improvement in environmental conditions.

Therefore, in response to specific HBV needlestick accidents, additional inoculation (once) of HB vaccine may be sufficient whether a patient is HBe antigen positive or HBe antibody positive. However, when HB vaccine has never been given, HB vaccine inoculation (three inoculations: at the time of the accident, 1 month later, and 3 months later) and intramuscular injection of HBIG (high-strength HBs immunoglobulin) should be given as soon as possible following the accident (without being restricted to the timeframe of 48 hours), as it has always been recommended. For post-accident follow-up, HBs antigen and ALT (GPT) should be tested once a month until six months following the accident.

2. Prevention of HCV infection

Several percent of the elderly population at least 60 years of age are HCV carriers, and only some of them have been identified as carriers. It is necessary to consider HCV to be present in the blood of the elderly.

There are no specific methods to prevent HCV infection as HBV infection is by HBIG or HB vaccines. However, it is a relief to know that HCV is less infectious than HBV.

Kiyosawa *et al.* were the first ones to report HCV infection caused by needlestick accidents.[4] Out of 200 needlestick accidents that happened to 196 healthcare workers, 107 cases of HCV infection were involved. Out of 110 accidents, three healthcare workers developed acute hepatitis (2.7%), and two other workers developed non-B non-C hepatitis. Since first generation HCV antibody tests were used at the time, it is possible that all five cases (4.5%) had acute hepatitis C.

Mitsui *et al.* have reported on HCV infection in healthcare workers that was caused by needlestick accidents in a dialysis center.[5] Out of 68 cases of accidents, seven cases (10%) developed acute hepatitis. Except for one case, they turned out to be a temporary infection.

Although there are many other reports on HCV infection caused by needlestick accidents, the incidence of the disease and the infection rate in HCV-exposed cases are not clear because not all of the needlestick accidents have been reported. The incidence of the disease is, therefore, assumed to be higher than the actual figures. An investigation in England has shown that only 1/3 of accidents is reported.

The low incidence of the actual establishment of HCV infection caused by needlestick accidents can also be surmised from many reports from various countries that show that there are no differences between the rate of positive HCV antibody among healthcare workers and that among blood donors.

As in the case of HBV infection, the use of commercially available immunoglobulin has been considered as a treatment option following needlestick accidents that may cause infection. Although administration of immunoglobulin was ineffective for prevention of the onset of HBV infection, prevention has become possible through immunoglobulin preparations containing a large quantity of HBs antibody. As for HCV, although there are antibodies that would prevent HCV infection, the protective antibody titer is expected to be much smaller compared with HBV. Therefore, a more condensed and specific immunoglobulin preparation needs to be developed.

Administration of interferon (INF) has also been attempted, but turned out to be ineffective. Since there is a several-hour timeframe before INF can manifest its effects in the body, there is a strong possibility that HCV that had slipped in during that time are attached to

hepatocytes. The rate for HCV infection to be established is low, and considering the adverse effects of INF, INF should not be used. In addition, even if acute hepatitis should occur, the possibility that it would heal is 30 to 40%. Moreover, if it is within one year of the onset of acute hepatitis, HCV can be successfully eliminated by INF at a high rate.[6]

Following HCV needlestick accidents, ALT and at times HC-RNA (qualitative) should be tested once a month for six months.

3. Prevention of HIV infection

The chances of getting infected with viruses as a result of needlestick accidents are found to be in the order of HBV, HCV, and HIV; the possibility of getting infected with HIV as a result of needlestick accidents is approximately 0.4%, the lowest of the three viruses. If the following preventive administration is started within 1–2 hours following the accident, the probability of infection reportedly drops to 1/5.[7]

When there is a possibility that the patient is an HIV carrier, the first preventive administration should be started even before it is confirmed by test results. When the patient is an HIV carrier or if test results have confirmed that this is the case, preventive administration should be started in both cases.

The following three drugs are used for preventive administration: Retrovir® 600 mg/day tid (after every meal), Epivir® 300 mg/day bid (after breakfast and supper), and Viracept® 2,250 mg/day tid (after every meal). After this preventive administration has been continued for four weeks, during which any adverse drug events should be carefully monitored, discontinue the administration. Verify that there is no HIV infection by conducting tests on the 6th week, the 12th week, the 6th month, and the 12th month. If HIV infection has been verified, begin treatment for HIV.

The primary adverse drug events of the three drugs are as follows:

- Retrovir®: Anemia, headache, malaise, fever, urticaria, gastrointestinal symptoms such as loss of appetite and nausea, mental symptoms such as dizziness and anxiety, respiratory symptoms, renal dysfunction, etc.
- Epivir®: Anemia, pancreatitis, neuropathy, confusion, seizure, heart failure, digestive symptoms, rash, etc.
- Viracept®: Diarrhea, rash, etc.

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＜特集関連情報＞

風疹のワクチン株と現在流行している野生株の遺伝子構造の比較

風疹ウイルス（rubella virus）のゲノムは9,757塩基から成る連続した一本のプラス鎖RNAで，5′端の約2/3は非構造タンパクをコードし，残りの約1/3はカプシドタンパクC，膜タンパクE1およびE2の3種類の構造タンパクをコードする。赤血球凝集能（HA）と膜融合にかかわる部位はE1にあり，中和にかかわる部位はE1とE2にある。E1，E2はともに膜表面に存在するが，抗原性を主に担っているのはE1である。

風疹ウイルスの遺伝子解析は1960年代以降の分離ウイルスについて主にE1遺伝子について行われてきた。風疹ウイルスはRNAウイルスであるにもかかわらず，年代を経ても遺伝子はあまり大きく変化していない。

1964～65年沖縄では風疹の大流行が起こり，多数の先天性風疹症候群（CRS）が発生した。その後1965～69年にかけて全国的に風疹の大流行が起こった。1971～74年に乾燥弱毒生風疹ワクチンが研究開発され，野外接種試験とマーカー試験に合格した5株が製造承認されたが，そのうちの1株は最近製造中止になった。これらのワクチンは1960年代に分離されたウイルスが親株になっており，現在まで同じワクチン株が使用されている（表1）。

これらのワクチン株が分離されてから約35年が経過しており，現在の流行株との抗原性の乖離の可能性が考えられたので，2001年，2002年，2003年の風疹ウイルス株のE1遺伝子（1,443塩基）の全塩基配列を決定して，推定されるE1ポリペプチド（481アミノ酸）のアミノ酸配列をワクチン株と比較した。また，これまでに加藤ら[1]により報告されている1995年までの日本の風疹ウイルス株のE1ポリペプチドとも比較した。

2001年のウイルス株はCRSの患者材料から，2002年の株は地域的な風疹流行における患者の咽頭ぬぐい液から，RK13細胞を用いて分離した。2003年の株は妊娠中に発疹を生じ，風疹ウイルスIgM抗体価が高かった患者の羊水からRT-PCRにより検出・増幅されたPCR産物から遺伝子配列を決定した。プライマーは，E2遺伝子の3′端からE1遺伝子の後に続く非コード領域の間を末端部分が重なった4個のフラグメントに分け，各々1st PCRとnested PCR用のプライマーを設定した（プライマーに関する情報は請求があればお教えします）。分離株には1st PCRを，羊水検体にはnested PCRを行い，PCR産物からABIキャピラリーシーケンサーを用いて直接塩基配列を決定した。2002年の分離株は4株すべてが同じ塩基配列をもっていたので，代表株だけを解析に用いた。

表2にワクチン株と過去の大流行時の代表株および現在の流行株の間の塩基配列およびアミノ酸配列のホモロジーを示した。日本の流行株のE1遺伝子の塩基配列は，ワクチン株と比較して95.7～98.0%のホモロジーを示した。2001，2002，2003年のウイルス株でもほぼ同様であった。一方，E1ポリペプチドのアミノ酸配列のホモロジーも日本の流行株との間で98.1～99.2%と高い数値を示しており，ワクチン株と現在の流行株の間にはE1ポリペプチドにほとんど変化が見られなかった。

E1タンパク上にはいくつかの抗原エピトープが存在するが，E1-195-296（102アミノ酸）の間に中和と赤血球凝集抑制（HI）に関する主な抗原領域が含まれていると報告されている[2]。このE1-195-296の間のアミノ酸配列については，1961年に米国で分離されたM33株由来のワクチン株HPV77と比べて，日本の株は0個～1個のアミノ酸の変化しかなかった。2002，

表1．日本の風しんワクチン

ウイルス株名	分離場所・年	継代歴*	ワクチン製造細胞*	製造機関
松浦	大阪・1966	GMK14 E65 Q11	Q	阪大微生物病研究会
To-336	富山・1967	GMK7 GPK20 RK3	RK	武田薬品工業株式会社
TCRB19**	東京・1967	GMK1 BK53 RK3	RK	千葉県血清研究所
高橋	松江・1968	GMK4 RT36 RK1	RK	北里研究所
松葉	熊本・1969	GMK3 SK60 RK11	RK	化学及血清療法研究所

*GMK：ミドリザル腎細胞、E：孵化鶏卵羊膜細胞、Q：ウズラ胚細胞、GPK：モルモット腎細胞、RK：ウサギ腎細胞、BK：牛腎細胞、RT：ウサギ睾丸細胞、SK：ブタ腎細胞、数字は継代歴
**現在は製造中止

表2．E1遺伝子およびE1ポリペプチド間のホモロジー

株　名	1	2	3	4	5	6	7	8	9	10
1 Matsuura 66		99.3	97.8	97.8	97.8	96.9	96.5	96.5	96.6	96.6
2 To-336 67	99.2		97.6	97.6	98.0	96.8	96.4	96.6	96.7	96.7
3 Takahashi 68	99.0	98.5		100	97.3	96.0	95.8	95.9	95.7	95.9
4 Matsuba 69	99.0	98.5	100		97.3	96.0	95.8	95.9	95.7	95.9
5 Akita 76	98.8	98.8	98.5	98.5		97.8	97.8	97.4	97.4	97.4
6 Ishikawa 87	98.3	98.3	98.3	98.3	98.8		96.8	97.2	97.3	97.3
7 Kumamoto 93	98.3	98.3	98.1	98.1	98.8	98.3		96.4	96.7	96.7
8 Miyazaki 2001	99.0	99.0	98.8	98.8	99.4	99.0	99.0		98.9	98.9
9 Okayama 2002	99.2	99.2	99.0	99.0	99.6	99.2	99.2	99.8		99.7
10 Hiroshima 2003	99.2	99.2	99.0	99.0	99.6	99.2	99.2	99.8	100	

下三角：アミノ酸配列の%ホモロジー、上三角：塩基配列の%ホモロジーを示す

2003年の流行株についても，To-336株および1991～95年の流行株と同様に，アミノ酸の変化が全く見られなかった。一方，ワクチンウイルス松浦株では219番目のアミノ酸がグリシン（G）からセリン（S）に置換しており，また，高橋株と松葉株とではE1の遺伝子配列は同一であり，いずれも203番目のアミノ酸がロイシン（L）からメチオニン（M）に置換していた。2001年の分離株は274番目のアミノ酸がバリン（V）からグルタミン酸（E）に変化していた。これらのアミノ酸の変化は株特異的な変化であり，それ以降のウイルスには同じ変化は引き継がれていなかった。

以上の結果から，1960年代の分離ウイルスに由来する4種類の日本のワクチン株と現在日本で流行している風疹ウイルスは，約35年もの開きがあるにもかかわらず，中和とHIにかかわる抗原エピトープ部位の遺伝子構造の乖離はほとんどなく，現行ワクチンが有効であることが推定される。

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＜特集関連情報＞

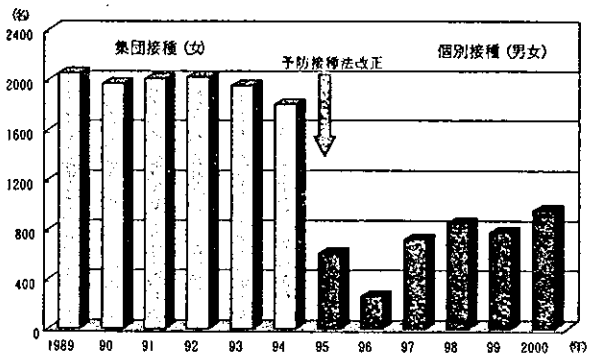
風疹ワクチンの接種率向上に対する対策――岡山県

予防接種法改正とその後の変化：風疹は，1995年まで5年周期で流行し，流行時に先天性風疹症候群（CRS）の発生が増加した。1965年～1985年の間にわが国で少なくとも1,600名のCRS児が生まれ，流行時には人工中絶も年間3～4万件増加したと推定されている。1995年予防接種法の改正により，風疹流行阻止によってCRSを防止しようと方針を変更した。風疹ワクチンの対象は女子中学生から，12～90カ月までの男女に変更になった。しかし，そのままでは90カ月～中学生までの児における接種機会がなくなるので，2003年まで暫定的に中学生男女も追加対象となった。また，集団接種から個別接種に変更となった。

生後12～90カ月の男女に接種となったため，接種率は約50～70％であるが免疫を有する者が急速に増加した。また麻疹などと比較すると感染力も弱いことから，流行がほとんどなくなっている。

一方，中学生は個別接種となったため，接種をしなくなった。我々が倉敷市で調査したところ，図に示すように改正前集団接種では約70％の接種率が1996年

図　倉敷市における中学生の風疹ワクチン接種者数



に約6％に著減した。この低下傾向は全国的なもので，中学生の接種率は全体で約40～50％であるが，まだ集団接種されているところが多く，都会で多い無料の個別接種では約20～30％しかない。日本産婦人科医会による2001（平成13）年妊婦の風疹抗体全国調査では，22～37歳までは4～6％の陰性率であったが，20～21歳で8％，18～19歳で13％，17歳以下で27％と年齢の低下とともに陰性率が増加していた。

将来の先天性風疹症候群の危惧と法見直しによる対策：予防接種法改正後，幼児の感受性者が急速に減少し流行はほとんどなくなった。幼児における1回接種の効果がブースターなしで成人まで持続するであろうか。また風疹は再感染することがよく知られており，再感染によってもCRSの発生が報告されている。風疹の流行がないと，結局接種率が抗体保有率となる。現在，成人女性における抗体保有率は約95％なので，今後も接種率が現状のままであると抗体保有率は低下する。接種率が大幅に改善しないと感受性者が次第に蓄積し，将来再び風疹の流行とCRSの増加を見ることになるであろう。

2001年11月に，暫定期間の未接種者は定期接種できるように予防接種法が見直された。しかし，医師を含めた多くの方はこの措置を知らない。我々の岡山県予防接種センター（川崎医大）では高校生以降の定期接種者が8カ月間でわずか3名，倉敷市でも99名しかいなかった。試算では多く見積もって感受性者の1.6％未満しかない。

暫定的対象者（中学生）に対する取り組み：1997年接種者数の著減に気づいたため，啓発活動を開始した。市保健課や教育委員会を通じて，広報誌による再度のお知らせや学校から保護者への連絡を実施し，地方新聞などでも啓発に努めた。また学校での啓発が重要と考えて，養護教諭や保護者を対象にした講演会を実施した。しかし，接種率は図のように満足な増加を得ることができなかった。保護者だけでなく，自分の意志を持つ中学生には直接啓発することも重要と考え，県健康対策課や教育委員会と協力して独自に作成した啓発用ビデオを岡山県内の中学校に送付した。視聴前後のアンケート調査では，ビデオ視聴した感受性者の

▷ウイルス検査の現状と問題点(2)◁

妊婦の風疹感染診断における抗体測定の現況と限界

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The Present Situation and Limitation of Antibody Assays for Diagnosis of Rubella Virus Infection in Pregnant Women

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Rubella virus infection during early stages of pregnancy often results in a number of developmental disorders referred to as congenital rubella syndrome (CRS). Both clinical and laboratory diagnosis of suspect cases of CRS can be made with relative ease, particularly when expectant mothers show the typical rubella-specific rash. Serological diagnosis of CRS is accomplished using hemagglutination inhibition (HI) and enzyme-linked immunosorbent (IgM-EIA) assays. Antibody titers as determined by these assays are generally very high following acute apparent rubella infections, thus making serological diagnosis relatively easy in most cases. However, the detection of possible CRS cases can be hampered by clinically inapparent rubella infections during early pregnancy. As much as 30 percent of all acute rubella cases are inapparent infections, and there is the very real potential for such inapparent infections to occur during pregnancy, to result in fetal infections, and consequently to cause CRS. Detection of CRS becomes extremely difficult in such settings. Complicating CRS detection even more are rare rubella re-infections that might occur in early pregnancy, and unknown risk of fetal infection and CRS. In re-infection cases, HI antibody titer becomes elevated due to a secondary immune response, and IgM antibody is produced in a significant number of cases.

To determine directly the fetal infection, virus genome detection was developed and applied clinically for the past decade. Using a combination of serological and genomic detection methods, the results of the investigation suggest that when rubella infection during early pregnancy occurs 1) there is a significant risk of fetal infection that results from acute apparent rubella infection, 2) there is a measurable risk of fetal infection resulting from inapparent infections as defined by HI antibody titers ≥ 256 and with an IgM-EIA index ≥ 7, and 3) high HI antibody titers with low IgM-EIA indices or no detectable IgM antibody in cases of inapparent rubella infections may represent rubella re-infections and result in a low risk of fetal infections.

[Rinsho Byori **51**：263～267, 2003]

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【**Key Words**】rubella(風疹), CRS(先天性風疹症候群), HI antibody(HI抗体), IgM-EIA, genome detection(遺伝子検出)

風疹は発疹を伴う全身性のウイルス感染症である。主として小児がかかる比較的軽い病気であるが，妊娠初期の妊婦が風疹にかかると，風疹ウイルスはその胎児に感染し，出生児に高率に先天性の難聴，白内障や心奇形などの障害(congenital rubella syndrome: CRS)を生じることが知られている。そのた

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め，小児の感染診断の場合と異なり，妊婦の急性感染の診断は，その結果が妊娠継続に影響を与えるので極めて重要である。

急性感染の診断法としては，血清中の風疹特異的抗体の測定が行われている。従来はペア血清の4倍以上の赤血球凝集抑制(HI)抗体価の上昇をもって急性感染と診断されてきたが，近年は酵素抗体法(EIA：enzyme-linked immunosorbent assay)によるIgM抗体の測定が主流になりつつある。一方，加藤らにより，胎児由来組織から風疹ウイルスの遺伝子を検出することによって胎児の感染の有無を調べる診断法が開発され，実施されてきた[1)6)]。

風疹の抗体価と遺伝子検出の両面から，妊婦の急性感染の診断における問題点を考察した。

I. 風疹特異抗体

風疹ウイルスは，内部に一本の＋鎖RNAを包むコア蛋白を有し，その外側が2種類の糖たんぱく質E1とE2を含む膜に包まれている。E1上には赤血球凝集(HA)活性とウイルスの感染性に関与する部位がある。風疹ウイルスに感染するとHA活性を抑制するHI抗体が産生され，その抗体価はIgG抗体として長期に維持される。HI抗体はウイルス中和抗体と相関していると考えられており，感染防御の目安となっている。他方，EIA抗体は，ウイルス抗原との結合性で測定される抗体であり，必ずしもHI活性や中和活性を持つとは限らない。IgM抗体はIgG抗体に先行して上昇し，3～4ヶ月でIgG抗体よりも早く消失する。したがって，IgM抗体の検出により推定される感染時期は，IgG抗体やペア血清のHI抗体価の変動から推測するよりも狭められ，急性感染の診断に適している。

A. HI抗体価の推移

顕性初感染の患者43人から経時的に採取した287検体のHI価は，発疹出現後1～2週間で256～8192倍に上昇し，最高値を示した[2)]。その後，HI価は少しずつ低下して，発疹7ヶ月後で多くは256倍になった。その後3年間は128倍のHI価が維持されていた。これらの結果から，512倍以上のHI価の場合，初感染後7ヶ月以内か，または再感染によって増強された抗体と推測された(**Fig. 1**)。妊婦の場合，妊娠10数週で抗体価が吟味されることが多いことから，急性感染の診断を行う場合を除いて，ペア血清のHI価の変動を調べることはHI価の上昇パターンから見ても実用的ではない。

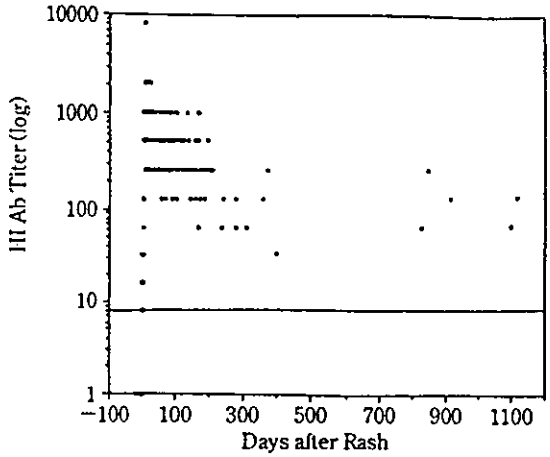

Figure 1 HI抗体価の推移[2)]
HI価＜1：8を陰性とする

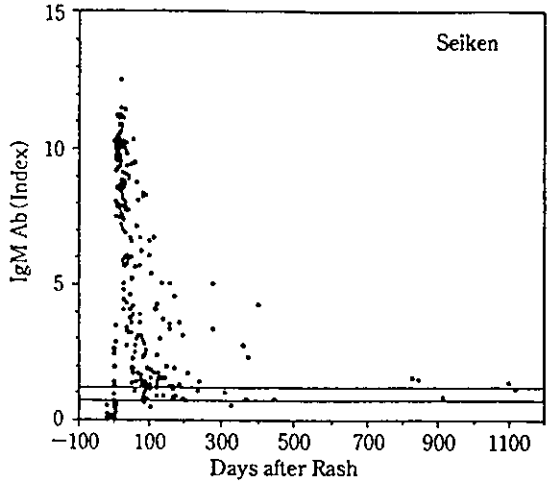

Figure 2 IgM抗体価の推移[2)]
図中上の横線が陽性境界値，下の横線が陰性境界値，両者の中間の値を疑陽性とする

B. IgM抗体の推移

国内で最も普及しているデンカ生研のEIAキットを用いて上記検体のIgM抗体指数を測定し，その結果を**Fig. 2**に示した。このキットでは，発疹後4日目から全ての検体のIgM抗体が陽性となったが，それ以前では陰性となる場合が見られた。その後，指数は，急上昇して6以上の高値に達した。指数は，発疹後50日目位から減少し始め，100日あたりで弱陽性値にまで下がった。しかし，300日でも中陽性を，1100日でも弱陽性を示す検体が認められた。こ