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23) Salcedo JR, Greenberg L, Kapur S. Renal histology of mucocutaneous lymph node syndrome (Kawasaki disease). Clin Nephrol. 1988; **29**: 47–51.
24) Tanaka T, Koike M, Minami Y. Pathology of liver injury in Kawasaki disease. Shoni-Naika. 1984; **16**: 2393–7. (in Japanese)
25) Ohshio G, Furukawa F, Fujiwara H, Hamashima Y. Hepatomegaly and splenomegaly in Kawasaki disease. Pediatr Pathol. 1985; **4**: 257–64.
26) Suddleson EA, Reid B, Woolley MM, Takahashi M. Hydrops of the gallbladder associated with Kawasaki syndrome. J Pediatr Surg. 1987; **22**: 956–9.
27) Masuda H, Naoe S, Tanaka N. pathological study on initial arterial lesions of Kawasaki disease, observation of removed gallbladders. Research committee of intractable vasculitis syndromes of the ministry of health and welfare of Japan, annual report for 1981, 1982: 274–9. (in Japanese)
28) Ando M, Asaji A, Naoe S, Masuda H, Tanaka N. Pathology of pancreatic arterial lesions in Kawasaki disease. Research committee of intractable vasculitis syndromes of the ministry of health and welfare of Japan, annual report for 1985, 1986: 117–22. (in Japanese)
29) Yoshioka H, Miyake T, Oshio G, Shimizu J, Takeushi E, Fujiwara K, et al. Clinicopathological study on the pancreas in 26 autopsy cases of Kawasaki disease. Journal of the society of Kinki area Kawasaki disease research, 1986; **8**: 30–3. (in Japanese)
30) Kurashige M, Naoe S, Masuda H, Tanaka N. A morphological study of the digestive tract in Kawasaki disease - 31 autopsies -. J Jap. Coll. Angiol. 1984, **24**: 407–18. (in Japanese)
31) Marsh WL Jr, Bishop JW, Koenig HM. Bone marrow and lymph node findings in a fatal case of Kawasaki's disease. Arch Pathol Lab Med. 1980, **104**: 563–7.
32) Giesker DW, Pastuszak WT, Forouhar FA, Krause PJ, Hine P. Lymph node biopsy for early diagnosis in Kawasaki disease. Am J Surg Pathol. 1982; **6**: 493–501.
33) Tanaka N, Naoe S, Masuda H, Ueno T. Pathological study of lymph node lesion in Kawasaki disease. Prog. Med. 1986; **6**: 21–7. (in Japanese)
34) Hirose S, Hamashima Y. Morphological observations on the vasculitis in the mucocutaneous lymphnode syndrome, A skin biopsy of 27 patients. Eur J Pediatr. 1978; **129**: 17–27.
35) Miyake K, Ota K, Kawamori J, Hirao T, Hamasaki Y. Histopathological findings of skin in acute and convalescent stage of patients with mucocutaneous lympnode syndrome. Igaku-no-ayumi. 1984; **128**: 805–6. (in Japanese)
36) Sugawara T, Hattori S, Hirose S, Furukawa S, Yabuta K, Shirai T. Immunopathology of the skin lesion of Kawasaki disease. Prog Clin Biol Res. 1987; **250**: 185–92.
37) Kuniyuki S, Asada M. An ulcerated lesion at BCG vaccination site during the course of Kawasaki disease. J Am Acad Dermatol. 1997; **37**: 303–4.
38) Sato N, Sagawa K, Sasaguri Y, Inoue O, Kato H. Immunopathology and cytokine detection in the skin lesions of patients with Kawasaki disease. J Pediatr. 1993; **122**: 198–203.
39) Masuda H, Shozawa T, Naoe S, Tanaka N. The intercostal artery in Kawasaki disease. A pathologic study of 17 autopsy cases. Arch Pathol Lab Med. 1986; **110**: 1136–42.
40) Burns JC, Wright JD, Newburger JW, Schneeberger EE, Mierau GW, Smith LE. Conjunctival biopsy in patients with Kawasaki disease. Pediatr Pathol Lab Med. 1995; **15**: 547–53.
41) Yoshioka H, Ohshio G, Takeuchi E, Hamashima Y. Severe inflammation of salivary glands and ducts in Kawasaki disease: A histopathological study of nine autopsy cases. Annales Paediatrici Japonici. 1986; **32**: 24–8.
42) Ko T, Fujiwara H, Shimizu J, Iwamoto H, Fujiwara K, Hamashima Y. Pathological study of testis in Kawasaki disease. Journal of the society of Kinki area Kawasaki disease research. 1983; **5**: 7–9. (in Japanese)
43) Amano S, Hazama F. Neutral involvement in Kawasaki disease. Acta Pathol Jpn. 1980; **30**: 365–73.
44) Rowley AH, Eckerley CA, JÃ¤ck HM, Shulman ST, Baker SC. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. J Immunol. 1997; **159**: 5946–55.
45) Rowley AH, Shulman ST, Mask CA, Finn LS, Terai M, Baker SC, et al. IgA plasma cell infiltration of proximal respiratory tract, pancreas, kidney, and coronary artery in acute Kawasaki disease. J Infect Dis. 2000; **182**: 1183–91.
46) Rowley AH, Shulman ST, Spike BT, Mask CA, Baker SC. Oligoclonal IgA response in the vascular wall in acute Kawasaki disease. J Immunol. 2001; **166**: 1334–43.
47) Rowley AH, Baker SC, Shulman ST, Garcia FL, Guzman-Cottrill JA, Chou P, et al. Detection of antigen in bronchial epithelium and macrophages in acute Kawasaki disease by use of synthetic antibody. J Infect Dis. 2004; **190**: 856–65.
48) Rowley AH, Baker SC, Shulman ST, Fox LM, Takahashi K, Garcia FL, et al. Cytoplasmic Inclusion Bodies Are Detected by Synthetic Antibody in Ciliated Bronchial Epithelium during Acute Kawasaki Disease. J Infect Dis. 2005; **192**: 1757–66.
49) Nagata S, Yamashiro Y, Maeda M, Ohtsuka Y, Yabuta K. Immunohistochemical studies on small intestinal mucosa in Kawasaki disease. Pediatr Res. 1993; **33**: 557–63.
50) Nagata S, Yamashiro Y, Ohtsuka Y, Shimizu T, Sakurai Y, Misawa S, et al. Heat shock proteins and superantigenic properties of bacteria from the gastrointestinal tract of patients with Kawasaki disease. Immunology. 2009; **128**: 511–20.

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BRIEF REPORT

Resolution of Henoch-Schönlein purpura nephritis after acquired IgA deficiency

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Abstract We report a case of Henoch-Schönlein purpura nephritis (HSPN) with acquired IgA deficiency due to parvovirus B19 infection. The patient was diagnosed as having Henoch-Schönlein purpura (HSP) at 6 years old, and subsequently developed macrohematuria and massive proteinuria of 7.4 g/day with decreased creatinine clearance of 70.2 ml/min/1.73 m^2 and significantly elevated serum IgA level of 449 mg/dl. The first kidney biopsy yielded the diagnosis of severe HSPN. After the initiation of the immunosuppressive therapy, the patient was infected with parvovirus B19 and developed virus-associated hemophagocytic syndrome (VAHS). Thereafter, the serum level of IgA selectively decreased and remained undetectable until the present time. Repeated kidney biopsies performed over a period of 14 years revealed a remarkable histological improvement in association with stabilization of the patient's kidney function. Considering the severity of initial kidney injury, persistent acquired IgA deficiency was likely to add favorable effects to the immunosuppressive therapy in this patient with HSPN.

Keywords Henoch-Schönlein purpura nephritis · IgA deficiency · Parvovirus B19 · Virus-associated hemophagocytic syndrome · IgA nephropathy

Introduction

Henoch-Schönlein purpura (HSP) is a generalized vasculitis characterized by cutaneous purpura, abdominal pain, arthritis, gastrointestinal bleeding, and nephritis [1]. Prognosis of most of the patients with Henoch-Schönlein purpura nephritis (HSPN) is relatively benign, but particularly those

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patients manifesting heavy proteinuria, rapidly progressive glomerulonephritis, or severely impaired glomerular structure with more than 75% of crescents have poor prognosis [1]. In addition, about half of the patients with HSPN grade V progress to end-stage kidney disease [1]. In this paper, we describe a case in which the initial severe kidney injury due to HSPN remarkably improved after acquired IgA deficiency with a long-term observation. This case may reinforce the importance of IgA antibody in the development and exacerbation of HSPN.

Case report

In October of 1993, a 6-year-old Japanese girl developed erythematous purpura on her legs, abdominal pain, and arthralgia. In April of 1994, after her 7th birthday, she was admitted to Juntendo University Hospital because of the development of massive proteinuria with macrohematuria. Upon admission, her blood pressure was 118/60 mmHg, and her body examinations were almost normal. Laboratory data showed serum creatinine concentration (SCr) of 0.8 mg/dl (70.7 μmol/l), creatinine clearance of 70.2 ml/min/1.73 m^2, significantly elevated serum IgA level of 449 mg/dl (age-related reference, 33–236 mg/dl), serum albumin of 3.8 g/dl, and proteinuria of 7.4 g/day. Serum test for antistreptolysin O, antistreptokinase, rheumatoid factor (RF), and antinuclear antibody (ANA) were negative, and serum complement level was slightly elevated. To investigate the cause of glomerulonephritis, the first open kidney biopsy was undertaken.

The kidney specimens revealed necrotizing crescentic glomerulonephritis (NCGN) with advanced glomerulosclerosis and severe tubulointerstitial fibrosis (Fig. 1a), which was compatible with HSPN grade V according to the criteria of International Study of Kidney Disease in Children (ISKDC) [2]. Diffuse global sclerosis was found in 36 of 49 glomeruli, and the rest of glomeruli showed segmental sclerosis and/or mesangial proliferation. Cellular crescents were found in nearly all the glomeruli, while fibrous or fibrocellular crescents were not found [normal glomeruli, 0/49 (0%)]. Tubulointerstitial inflammation and fibrosis were very severe. Although immunofluorescence (IF) and electron microscopic examination were not performed, we diagnosed HSPN with clinical and histological findings, and started immunosuppressive therapy including prednisolone (PSL), azathioprine (AZP), warfarin, and dipyridamole.

During this treatment, the patient presented rush on each cheek followed by rapid expansion to trunk and extremities, and infectious erythema caused by parvovirus B19 was suspected clinically. A few days later, she also developed a high fever with agranulocytosis, mild anemia, and severe hypertriglyceridemia. Bone-marrow specimen showed hemophagocytosis. These histories were compatible with virus-associated hemophagocytic syndrome (VAHS) due to parvovirus B19 infection [3].

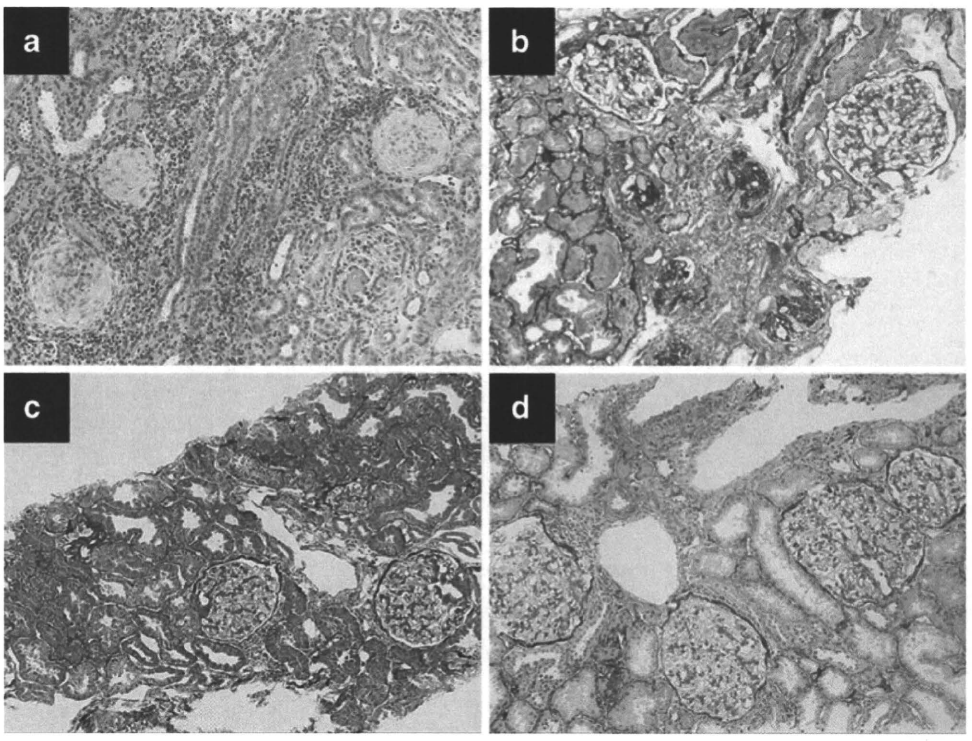

Fig. 1 Repeated kidney biopsies showed significant histological improvement through a period of 14 years. **a** The first biopsy revealed necrotizing crescentic glomerulonephritis with advanced glomerulosclerosis and severe tubulointerstitial nephritis (hematoxylin-eosin staining; original magnification × 40). **b** The second biopsy revealed focal glomerulosclerosis without any crescent (methenamine silver staining; original magnification×40). **c** The third biopsy showed further improvement with focal glomerular obsolescence (methenamine silver staining; original magnification×40). **d** The fourth biopsy revealed minor glomerular abnormalities (methenamine silver staining; original magnification×40)

When she suffered from VAHS, she had transient serum IgM elevation to 396 mg/dl (age-related reference, 43–207 mg/dl). However, after she eventually recovered from VAHS, serum IgA declined to an undetectable range while serum IgM was normalized. Then, her kidney function was gradually normalized after 6 months of treatment, and complete remission of proteinuria was achieved after another 6 months (1995, March).

However, in July of 1996, when she was 9 years old, proteinuria increased to 0.64 g/day. Her SCr was 0.4 mg/dl (35.4 μmol/l) and serum IgA was less than 7 mg/dl (undetectable). The second open-kidney biopsy was performed at Tokyo Metropolitan Kiyose Children's Hospital to determine the management of HSPN. It revealed a significant reduction in the proportion of sclerotic lesions without any crescents (Fig. 1b). The kidney tissue contained 120 glomeruli, 47 of which were globally sclerosed. Five glomeruli showed segmental sclerosis, however, the remaining 68 glomeruli were almost normal [normal glomeruli, 68/120 (56%)]. Then, the therapy with angiotensin-converting enzyme inhibitor was started, and proteinuria gradually decreased to less than the normal limit (1998, April).

In August 2001, the third needle kidney biopsy, performed at 13 years old for the relapse of slight proteinuria with hematuria, showed further histological improvement (Fig. 1c). A total of 23 glomeruli were obtained, and only two of which were globally sclerosed. The other glomeruli were almost normal and no crescents were seen [normal glomeruli, 21/23 (91%)]. At this time, her SCr was 0.7 mg/dl (61.9 μmol/l), proteinuria was 0.36 g/day, and serum IgA was less than 3.5 mg/dl (undetectable).

In 2008, at 21 years old, she was admitted to The University of Tokyo Hospital for kidney biopsy, which was indicated to examine her risks of future pregnancy. Physical examinations were normal except for her swollen tonsils. Her blood pressure remained normal, SCr was 0.8 mg/dl (70.7 μmol/l), estimated glomerular filtration rate was 94.2 ml/min/1.73 m^2, and proteinuria was 0.29 g/day. Serum IgA was still less than 10 mg/dl (undetectable). Serum test for RF, ANA, antineutrophil cytoplasmic antibodies (ANCA), and cryoglobulin were all negative. The fourth needle kidney biopsy, containing of 13 glomeruli, revealed minor glomerular abnormalities with compensatory glomerular and tubular hypertrophy, which were compatible with chronic inactive phase of NCGN (Fig. 1d). There were no sclerotic glomerular lesions or crescents [normal glomeruli, 13/13 (100%)]. In the second, third, and fourth kidney biopsies, IF examination was negative for IgA staining and electron microscopy revealed no electron dense deposit. These histological improvements seemed to correlate well with the patient's kidney function.

Discussion

Acquired IgA deficiency is rare, but parvovirus B19 infections are known to lead to acquired immunodeficiency associated with hemophagocytic syndrome [3]. Gleeson et al. reported a rare case of persistent acquired IgA deficiency after respiratory infection probably due to Epstein–Barr virus (EBV) [4]. The authors found a transient elevation in saliva IgM preceding the onset of IgA deficiency, and a similar trend was also observed in the serum samples of our patient. This phenomenon suggested that a viral infection and/or subsequent VAHS affected B lymphocytes and prevented class switch recombination from the IgM class to IgA.

Because of some technical reasons, IF examinations including IgA antibody were not performed at the first biopsy. However, a combination of clinical manifestations, high serum IgA level, and subsequent development of NCGN supported the diagnosis of HSPN. Other kidney diseases, such as lupus nephritis and post-infectious glomerulonephritis, which could present multiple organ involvement including NCGN with nephrotic-range proteinuria and macrohematuria, were all excluded clinically, serologically, or histologically. Considering the histological findings, the cause of ongoing slight proteinuria after IgA deficiency might be subsequent glomerular hyperfiltration.

Previously, Iijima et al. demonstrated the efficacy of multiple combined therapy with PSL, cyclophosphamide, heparin/warfarin, and dipyridamole in severe HSPN of ISKDC grade IV or V [5]. Although histological grade was significantly improved after the combined therapy, patients achieved grade III at best in that study. The multiple combined therapy with PSL, AZP, heparin/warfarin, and dipyridamole was also reported to be effective in childhood IgA nephropathy (IgAN), which is now regarded as a related disease to HSPN, but the percentage of glomeruli showing sclerosis was unchanged [6]. We cannot exclude the possibility that the inevitable sampling variations among repeated kidney biopsies might have somehow overestimated the histological improvement in the present case. Nevertheless, these considerations strongly suggest that the immunosuppressive therapy alone was unlikely to be responsible for the excellent clinical course. It is rather tempting to speculate that acquired IgA deficiency caused either by infectious erythema or VAHS might work quite favorably on the present case.

The resolution of IgAN is sometimes associated with the vanishment of IgA deposition from glomerular regions [7]. Lamm et al. reported that the systemically administered IgA protease was able to remove glomerular IgA immune complexes in a mouse model of IgAN [8]. Future studies are required to determine whether such a therapy aiming to remove glomerular IgA immune complexes is effective for human IgA-related diseases.

References

1. Schärer K, Krmar R, Querfeld U, Ruder H, Waldherr R, Schaefer F (1999) Clinical outcome of Schönlein-Henoch purpura nephritis in children. Pediatr Nephrol 13:816–823
2. International Study of Kidney Disease in Children (1978) Nephrotic syndrome in children: prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. Kidney Int 13:159–165
3. Créput C, Galicier L, Buyse S, Azoulay E (2008) Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. Intensive Care Med 34:1177–1187
4. Gleeson M, Clancy RL, Cripps AW, Henry RL, Hensley MJ, Wlodarczyk JH (1994) Acquired IgA deficiency. Pediatr Allergy Immunol 5:157–161
5. Iijima K, Ito-Kariya S, Nakamura H, Yoshikawa N (1998) Multiple combined therapy for severe Henoch-Schönlein nephritis in children. Pediatr Nephrol 12:244–248
6. Yoshikawa N, Ito H, Sakai T, Takekoshi Y, Honda M, Awazu M, Ito K, Iitaka K, Koitabashi Y, Yamaoka K, Nakagawa K, Nakamura H, Matsuyama S, Seino Y, Takeda N, Hattori S, Ninomiya M (1999) A controlled trial of combined therapy for newly diagnosed severe childhood IgA nephropathy. The Japanese Pediatric IgA Nephropathy Treatment Study Group. J Am Soc Nephrol 10:101–109
7. Yoshikawa N, Iijima K, Matsuyama S, Suzuki J, Kameda A, Okada S, Nakamura H (1990) Repeat renal biopsy in children with IgA nephropathy. Clin Nephrol 33:160–167
8. Lamm ME, Emancipator SN, Robinson JK, Yamashita M, Fujioka H, Qiu J, Plaut AG (2008) Microbial IgA protease removes IgA immune complexes from mouse glomeruli in vivo: potential therapy for IgA nephropathy. Am J Pathol 172:31–36

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Association Between Capacity of Interferon-α Production and Metabolic Parameters

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A prompt and regulated interferon (IFN) system is critical for host defense against infectious pathogens. Although increased susceptibility to infection has been observed in subjects with diabetes or obesity, little is known about the relationship between metabolic disorders and increased susceptibility to infection. In order to evaluate the association between immune function and metabolic parameters, we examined the relationship between capacity of IFN-α production and metabolic parameters including fasting plasma glucose (FPG), lipids, uric acid, body mass index (BMI), and blood pressure in 575 healthy subjects. Linear regression analysis showed that log(IFN-α production) was positively correlated with log(triglyceride) ($r = 0.088$, $P = 0.03$) and uric acid ($r = 0.091$, $P = 0.03$), and negatively correlated with age ($r = -0.158$, $P = 0.0001$) and FPG ($r = -0.088$, $P = 0.03$). Multiple regression analysis showed that log(IFN-α production) was independently determined by age ($\beta = -0.148$, $P < 0.0001$), sex ($\beta = -0.240$, $P = 0.0003$), and FPG ($\beta = -0.096$, $P = 0.0209$), suggesting that lesser degrees of hyperglycemia also affect IFN-α production. We conclude that hyperglycemia but not BMI, hypertension, or hyperlipidemia may be associated with decreased capacity of IFN-α production and glycemic control is critical even for both subjects without any medication for diabetes and subjects under the diagnosis of diabetes on infectious diseases.

Introduction

IT IS WIDELY KNOWN that individuals with diabetes mellitus are at higher risk for infections than those without diabetes mellitus. A higher prevalence of bacteriuria has been observed in subjects with diabetes compared with normal women (19% versus 8%) (Vejlsgaard 1966). In the last decade, several epidemiological studies have found an association between obesity and an increased incidence of periodontal disease (Al-Zahrani and others 2003; Genco and others 2005; Saito and others 2005; Saito and others 1998). It was reported that a higher body mass index (BMI) was significantly related to a greater prevalence of periodontal disease in Japanese healthy adults (Saito and others 2001). These observations may suggest a relationship between metabolic disorders and increased susceptibility to infection.

Interferon-α (IFN-α) is known to play an important role in the initial defense mechanism against viral and microbial diseases (Kirchner 1986). Indeed, functional immunity can be monitored using *in vitro* tests, such as natural killer cell activity and either proliferation responses or cytokine induction in lymphoid cells following mitogen stimulation. However, these measurements are found to reflect the function of natural killer cell or T cell (Biron and Brossay 2001; Stone and others 2009). As an initial response to viral and microbial infection, plasmacytoid dendritic cells (pDCs), also called type I IFN-producing cells (IPCs), is known to be central to the innate immune response of a host (Liu 2005). PDCs produce enormous amounts of IFN-α, which indirectly regulates the function of T cells and thus links innate and adaptive immune responses (Asselin-Paturel and others 2005; Fitzgerald-Bocarsly and Feng 2007). In fact, such prompt and regulated IFN system is critical for host defense against infectious pathogens. We therefore measured capacity of IFN-α production to assess the immune status of the host.

To further evaluate the association between immune function and various metabolic parameters, we examined the relationship between capacity of IFN-α production and

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fasting plasma glucose (FPG), lipids, uric acid, BMI, and blood pressure.

Materials and Methods

Subjects

Medical records of 877 individuals aged 20–79 years old, who participated in annual health examinations at 2 workplaces in Kyoto, Japan were obtained. The subject records included data on the quantification of IFN-α production in addition to routine laboratory tests. Data from the Louis Pasteur Center for Medical Research (n = 699) were reviewed from 1996 to 2004 records, and data from the Kyoto First Red Cross Hospital (n = 178) were reviewed from 2005 records.

All subjects were studied in the morning after 8 h of overnight fasting. Measurements were taken of FPG, lipid profile, uric acid, and leukocyte count, which were included in the routine laboratory blood test. BMI was calculated by the patient's weight (kilograms) divided by height (square meters). Blood pressure was measured in the patient's right arm, with the subjects in a sitting position.

After exclusion of 66 subjects with missing samples, subjects with acute infection (n = 17), chronic inflammatory disease (rheumatoid arthritis n = 15, autoimmune diseases n = 22, chronic hepatitis n = 71), and malignant tumors (n = 9) were excluded. Those who received medication for diabetes mellitus, hypertension, hyperlipidemia, or hyperuricemia (n = 102) were also excluded. Finally, a total of 575 subjects were recruited into this study. We investigated the relationships of IFN-α production to FPG, lipids, uric acid, BMI, and blood pressure. Approval for the study had been provided by the Ethics Committee of Louis Pasteur Center for Medical Research and Kyoto First Red Cross Hospital. All participants gave written informed consent.

Measurement of IFN-α production in human whole blood

Two milliliters of heparinized blood was cultured with 500 HA U/mL Sendai virus (HVJ) within 5 h after the blood was drawn. The blood–virus mixture was incubated at 37°C for 20 h. Supernatants were harvested by centrifugation at 3,000 rpm for 10 min and stored at −80°C until titration.

Table 1. Clinical Characteristics of the Subjects

	Mean ± SE
n	575
Age (years)	52.0 ± 0.4
IFN-α production (IU/mL)	7,237.4 ± 226.0
Systolic blood pressure (mmHg)	123.6 ± 0.7
Diastolic blood pressure (mmHg)	75.6 ± 0.4
BMI (kg/m^2)	22.6 ± 0.1
Fasting plasma glucose (mg/dL)	97.4 ± 0.9
Total cholesterol (mg/dL)	207.5 ± 1.5
Triglyceride (mg/dL)	121.0 ± 4.1
HDL-cholesterol (mg/dL)	57.9 ± 0.6
Uric acid (mg/dL)	5.2 ± 0.05

BMI, body mass index; HDL, high-density lipoprotein; IFN, interferon.

IFN activity in the supernatants was assayed by suppression of viral cytopathic effects in FL cells (derived from human amnion) by Sindbis virus (Kohase and others 1986). Duplicate samples of standard reference IFN were serially diluted by Eagles' MEM supplemented with 5% FCS in the wells of flat-bottomed 96-well microtiter plates (Falcon 3072) and irradiated with UV light (1,000 J/m^2) for HVJ inactivation. After UV treatment, HVJ in the blood samples could not affect the assay. FL cells (5×10^4/well) were added following incubation at 37°C for 18 h. Sindbis virus (10^5 PFU/50 μL/well) suspended in Eagles' MEM supplemented with 1% FCS was added to the FL cells after removal of the supernatant. Sindbis virus and FL cells were cultured for another 30 h. Neutral red dye (0.5%) was added 1 h before harvesting, and the amount of dye incorporated into the cells was measured in a spectrophotometer (Titertek Multiskan MCC/340) at 540 nm following extraction of dye with 0.1 M sodium phosphate and 50% ethanol solution. The results were standardized to reference IFN-α MRC 69/19B. For the characterization of IFN type, some samples were neutralized by anti-human IFN-α horse IgG (Nippon Chemical Co. Ltd., Hyougo) as described previously (Shirono and others 1990; Kuo and others 1991).

Statistical analysis

The statistical analysis was mainly performed by parametric tests. Thus, several variables with asymmetric distribution to the right were logarithmically transformed (base 10) in order to obtain substantially normal distributions before performing the tests. The log-transformed variables were IFN-α production, triglyceride. According to the analysis, male subjects were assigned 1 and female subjects were assigned 2. To describe the variables measured in this study, mean ± SE was used. Statistical analyses were conducted using the Stat View 5 system (Stat View, Berkeley, CA).

Linear regression analysis was performed to examine the relationships between IFN-α production and other variables, and also to exclude any significant collinearity among the variables before performing multivariable analysis. Multiple regression analysis was performed to assess the combined influence of variables on IFN-α production. P values <0.05 was considered as significant.

Results

Clinical characteristics of the subjects are shown in Table 1. Mean value of IFN-α production was 7,237.4 ± 226.0 IU/mL. In male subjects, the mean value of IFN-α production was 8,278.5 ± 334.0 IU/mL and female 5,884.0 ± 262.7 IU/mL, respectively. There was a significant difference between men and women (P = 0.0003). Pearson's correlation coefficients between log(IFN-α production) and other variables are shown in Table 2. There were statistically significant positive correlations between log(IFN-α production) and log(TG) (r = 0.088, P = 0.03), uric acid (r = 0.091, P = 0.03). Negative correlations were found between log(IFN-α production) and age (r = −0.158, P = 0.0001), FPG (r =−0.088, P = 0.03). No significant correlation was found between log(IFN-α production) and BMI.

Multiple linear regression analysis performed with log(IFN-α production) as the dependent variable and the candidate factors (age, sex, FPG, log(TG), uric acid) as the independent variables showed that age (β = −0.148,

TABLE 2. CORRELATION OF log(IFN-α PRODUCTION) TO MEASURES OF VARIABLES

	r	P
Age	−0.158	0.0001
Systolic blood pressure	0.04	0.34
Diastolic blood pressure	0.078	0.06
BMI	0.044	0.29
Fasting plasma glucose	−0.088	0.03
Total cholesterol	−0.028	0.5
Log(triglyceride)	0.088	0.03
HDL-cholesterol	−0.078	0.06
Uric acid	0.091	0.03

BMI, body mass index; HDL, high-density lipoprotein; IFN, interferon.

TABLE 3. MULTIPLE REGRESSION ANALYSIS ON log(IFN-α PRODUCTION)

	β	P
Age	−0.148	<0.0001
Sex	−0.240	0.0003
Fasting plasma glucose	−0.096	0.0209
Log(triglyceride)	0.057	0.2065
Uric acid	−0.054	0.2935

β, Standardized coefficients; IFN, interferon.

$P < 0.0001$), sex ($\beta = -0.240$, $P = 0.0003$), and FPG ($\beta = -0.096$, $P = 0.0209$) were independent determinants of log(IFN-α production) (Table 3).

Furthermore, we determined IFN-α production trend in subjects who had a history of health examination for a long period at the Louis Pasteur Center for Medical Research to examine a correlation among each individual. Among 64 subjects with abnormal FPG levels (≥110 mg/dL), 10 had their IFN-α production monitored once or twice per year for >5 years. During follow-up, 3 subjects developed diabetes and 7 showed increase in FPG levels, though below the diagnosis of diabetes. Although IFN-α production showed fluctuation, a simple regression line with the IFN-α production and age yielded a negative slope, while FPG levels showed increase, in all 10 subjects. The representative profile of one subject is shown (Fig. 1).

Discussion

In this study, we demonstrated that capacity of IFN-α production correlated significantly with age, sex, and FPG, but not with BMI, blood pressure, uric acid, or lipids.

Age is known to play a role in the regulation of IFN-α production, which is followed by a gradual decline with increased age (Abb and others 1984; Kita and others 1991; Katschinski and others 1994). This is consistent with the finding of Shodell et al. who reported significant decreases of the circulating pDCs in healthy aged humans, as was defined both by flow cytometry and IFN-α production (Shodell and Siegal 2002). There was also a small decline in the amount of IFN produced per pDC over the entire age range. On the other hand, no change in the total lymphocyte or monocyte counts was observed. Thus the age-related losses of IFN-α production may be due to both declining pDC numbers and a small reduction in IFN produced per pDC with aging.

Although previous studies showed no sex difference (Abb and others 1984; Kita and others 1991; Katschinski and others 1994), we observed decreased production of IFN-α in females in this study. It has been reported that the pDC numbers were not different between males and females (Shodell and Siegal 2002; Berghöfer and others 2006). Other factors like differences in innate immunity between males and females or systemic imposition of sex hormones may play a role; however, the mechanisms are not well understood (Kovats and Carreras 2008). To address these questions, further studies are required. FPG revealed to be another important determinant of IFN-α production, suggesting that lesser degrees of hyperglycemia also affect IFN-α production. And this was also observed in the history of long-term follow-up of IFN-α production among the 10 individuals with abnormal FPG levels.

Our data may coincide with a recent report by Summers et al. who described a reduced secretion of IFN-α by dentritic cells (DC) in both type 1 and type 2 diabetes (Summers and

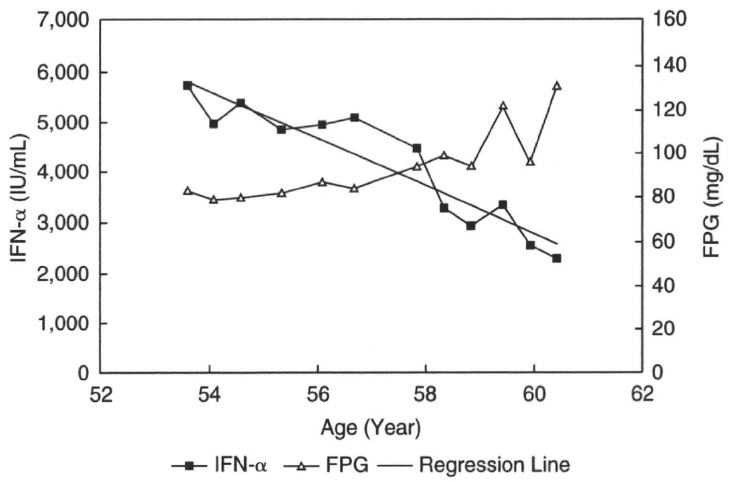

FIG. 1. Interferon-α (IFN-α) production trend in subjects with elevated fasting plasma glucose (FPG) levels, though under diagnosis of diabetes. The IFN-α values and FPG levels are plotted versus time.

others 2006). In their study, DC subsets in each diabetic group exhibit normal properties concerning frequency and activation state. Only low secretion of IFN-α was observed. However, the number of type 2 diabetes patients examined were quite low ($n = 7$), and the degree of glycemic control was not evaluated in their studies. Seifarth et al. reported significantly lower absolute numbers of pDC in patients with type 2 diabetes under poor glycemic control ($HbA_{1c} > 9.5\%$) compared to healthy controls (Seifarth and others 2008). Reduced absolute numbers of pDC in patients with type 2 diabetes under good glycemic control ($HbA_{1c} < 7.0\%$) was also observed compared with healthy controls, but this difference did not reach statistical significance (Seifarth and others 2008). The reason for this reduction in peripheral immune cells remains unclear; however, the decrease in the main IFN-producing cell counts may be a possible explanation of the decline in IFN-α production.

Although leukocyte count also showed correlation with IFN-α production, we did not adjust IFN-α production with leukocyte count. In this study, we aimed to investigate the immune status of each individual, but not the function of leukocyte. Moreover, the main IFN-α producer is pDC and we considered it inappropriate to overestimate the influence of leukocyte count.

There are some limitations to this study. In a bioassay of IFN, we cannot distinguish the subtypes of IFN induced by Sendai virus yet known as a potent IFN-α inducer (Klein and others 1984). However, the main subtype of IFN induced in whole blood cultures is probably IFN-α, as suggested by the results of neutralization experiments with anti-natural IFN-α antibodies, which are similar to the results of our previous study (Kuo and others 1991). As we considered it more important to determine the active forms of IFN than the subtype of IFN to prevent virus infection, titer of IFN using a bioassay of whole blood cultures was measured.

In conclusion, hyperglycemia but not BMI, hypertension, or hyperlipidemia may be associated with decrease in capacity of IFN-α production. Lesser degrees of hyperglycemia also affect IFN-α production, suggesting that glycemic control is critical even for both subjects without any medication for diabetes and subjects under the diagnosis of diabetes on infectious diseases.

Author Disclosure Statement

No competing financial interests exist.

References

Abb J, Abb H, Deinhardt F. 1984. Age-related decline of human interferon alpha and interferon gamma production. Blut 48(5):285–289.

Al-Zahrani MS, Bissada NF, Borawskit EA. 2003. Obesity and periodontal disease in young, middle-aged, and older adults. J Periodontol 74(5):610–615.

Asselin-Paturel C, Brizard G, Chemin K, Boonstra A, O'Garra A, Vicari A, Trinchieri G. 2005. Type I interferon dependence of plasmacytoid dendritic cell activation and migration. J Exp Med 201(7):1157–1167.

Berghöfer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H. 2006. TLR7 ligands induce higher IFN-alpha production in females. J Immunol 177(4):2088–2096.

Biron CA, Brossay L. 2001. NK cells and NKT cells in innate defense against viral infections. Curr Opin Immunol 13(4):458–464.

Fitzgerald-Bocarsly P, Feng D. 2007. The role of type I interferon production by dendritic cells in host defense. Biochimie 89(6-7):843–855.

Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y. 2005. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. J Periodontol 76(11 Suppl):2075–2084.

Katschinski DM, Neustock P, Klüter H, Kirchner H. 1994. Influence of various factors on interferon-alpha production in cultures of human leukocytes. J Interferon Res 14(3):105–110.

Kirchner H. 1986. The interferon system as an integral part of the defense system against infections. Antiviral Res 6(1):1–17.

Kita M, Shiozawa S, Yamaji M, Kitoh I, Kishida T. 1991. Production of human alpha- and gamma-interferon is dependent on age and sex and is decreased in rheumatoid arthritis: a simple method for a large-scale assay. J Clin Lab Anal 5(4):238–241.

Klein F, Ricketts RT, Jones WI, Clark P. 1984. Induction potential of Sendai virus and Newcastle disease virus for human lymphoblastoid interferon production. J Interferon Res 4(2):243–247.

Kohase M, Moriya H, Sato TA, Kohno S, Yamazaki S. 1986. Purification and characterization of chick interferon induced by viruses. J Gen Virol 67 (Pt 1):215–218.

Kovats S, Carreras E. 2008. Regulation of dendritic cell differentiation and function by estrogen receptor ligands. Cell Immunol 252(1-2):81–90.

Kuo JY, Nishio Y, Kanamaru H, Okada Y, Kita M, Imanishi J, Kishida T, Yoshida O. 1991. Impaired interferon-alpha production in whole-blood cultures from bladder cancer patients. Urol Res 19(1):51–56.

Liu YJ. 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 23:275–306.

Saito T, Shimazaki Y, Kiyohara Y, Kato I, Kubo M, Iida M, Yamashita Y. 2005. Relationship between obesity, glucose tolerance, and periodontal disease in Japanese women: the Hisayama study. J Periodont Res 40(4):346–353.

Saito T, Shimazaki Y, Koga T, Tsuzuki M, Ohshima A. 2001. Relationship between upper body obesity and periodontitis. J Dent Res 80(7):1631–1636.

Saito T, Shimazaki Y, Sakamoto M. 1998. Obesity and periodontitis. N Engl J Med 339(7):482–483.

Seifarth CC, Hinkmann C, Hahn EG, Lohmann T, Harsch IA. 2008. Reduced frequency of peripheral dendritic cells in type 2 diabetes. Exp Clin Endocrinol Diabetes 116(3):162–166.

Shirono H, Ito C, Koga J. 1990. Studies on subtype composition in natural leukocyte interferon preparations. J Virol Methods 27(1):1–9.

Shodell M, Siegal FP. 2002. Circulating, interferon-producing plasmacytoid dendritic cells decline during human ageing. Scand J Immunol 56(5):518–521.

Stone KD, Feldman HA, Huisman C, Howlett C, Jabara HH, Bonilla FA. 2009. Analysis of *in vitro* lymphocyte proliferation as a screening tool for cellular immunodeficiency. Clin Immunol 131(1):41–49.

Summers KL, Marleau AM, Mahon JL, McManus R, Hramiak I, Singh B. 2006. Reduced IFN-alpha secretion by blood dendritic cells in human diabetes. Clin Immunol 121(1):81–89.

Vejlsgaard R. 1966. Studies on urinary infection in diabetics. I. Bacteriuria in patients with diabetes mellitus and in control subjects. Acta Med Scand 179(2):173–182.No competing financial interests exist.

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ORIGINAL ARTICLE

Pre-operative intracellular glutathione levels of peripheral monocytes as a biomarker to predict survival of colorectal cancer patients

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Abstract The ability to predict anti-tumor immune responses at local tumor growing sites using only peripheral blood specimens would be helpful in determining therapeutic options for patients with solid tumors. Here, we show that the glutathione intracellular content (icGSH) of peripheral monocytes (Mo) correlates positively with T cell infiltration within tumor islets and overall survival in patients with colorectal carcinoma. IcGSH redox status was determined in $CD14^+$ Mo prior to surgery by staining with monochlorobimane. The tumor-infiltrating T cells (TIL) were quantified as $CD45RO^+$ T cells in resected tumors using paraffin sections. A positive association was found between the GSH index and TIL in tumor islets ($P < 0.001$). The 50% cut-off value for the GSH index, that is the determinant between TIL presence or absence in tumor islets, was calculated to be almost 0.7 through logistic regression analysis. Mo with a GSH index of ≥ 0.7 were termed *reductive* (R)-Mo, and those with <0.7 were designated as *oxidative* (O)-Mo. Cox's proportional hazards regression analysis of patients with R-Mo or O-Mo prior to surgery, and the presence or absence of TIL, was found to correlate significantly with the overall survival rate of stage II and III patients. Kaplan–Meier analysis also showed a significant correlation. These results indicate that the Mo icGSH index is a useful biomarker parameter for better understanding the host/tumor relationship prior to surgery, thereby enabling the development of an individual patient-oriented therapeutic strategy.

Keywords Glutathione · Reductive monocyte · Oxidative monocyte · Tumor infiltrate T cell · Colorectal cancer

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Introduction

The goal of this research is to improve the survival rate of patients with solid tumors who undergo anti-tumor therapy. To this end, we explored the possibility of predicting local anti-tumor reactions prior to surgery using biomarkers easily measurable in peripheral blood specimens. Such predictions will be helpful in determining therapeutic options for patients with solid tumors and more importantly allow for the optimal administration of pre-surgery treatments. Here, we explore whether studying peripheral

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monocytes (Mo) can in fact allow us to make such useful predictions. As Mo are recruited from the circulation and differentiate into mature macrophages (Mf) within the tumor micro-environment, they affect the constitution of the tumor stroma, resulting in a distinctive inflammatory condition, and may thus crucially influence the clinical outcome [1, 2]. This is supported by Galon et al. who demonstrated the importance of the adaptive immunologic micro-environment, especially the presence of TIL, to the clinical outcome in colorectal cancer patients [3, 4].

Hamuro et al. [5] proposed the functional discrimination of two classes of Mf; R-Mf with a high intracellular glutathione (icGSH), and O-Mf with a reduced content. They demonstrated that the Th1/Th2 ratio was regulated by the balance between R-Mf and O-Mf. This balance was associated with a difference in IL-12 versus IL-10 production, which further affects the development of the tumor stroma area [5, 6]. These findings are consistent with those of Peterson et al. [7], who also demonstrated that levels in antigen-presenting cells modulate Th1 and Th2 response patterns. These results suggest that R-Mo positively affects and induce anti-tumor function that may improve overall survival of cancer patients.

In this paper, we determined the relationship between the presence of TIL in tumor cell islets in resected tumors and icGSH levels of peripheral Mo in patients with colorectal cancer before surgery. The correlation between these two parameters was found and then correlated with overall patient survival after surgery. In this way, we found evidence that among the Mo-GSH index, the presence of TIL in tumor islets and the prognosis for colorectal carcinoma patients were positively correlated. These results demonstrate that the intracellular redox status monitored by icGSH levels in Mo/Mf plays a crucial role in the development of the anti-tumor response, therefore determining of icGSH has great potential as a favorable prognostic biomarker parameter in survival prediction.

Table 1 Patient characteristics

	No. of patients
Total	30
Male/female	21/9
Age (mean ± SD)	63.4 ± 1.71 years
Tumor types	
Colon	11
Rectum	19
Metastasis	
Localized Ca.	25
Meta.	5
Histological type	
Well differentiated	8
Moderately differentiated	21
Poorly differentiated	1
Tumor stage (GSH index: ≧0.7/<0.7)	
Stage 0, I	2 (1/1)
Stage II	9 (6/3)
Stage IIIa, b	14 (11/3)
Stage IV	5 (5/0)
GSH index	
Total	30 (23/7)
Mean ± SD	0.92 ± 0.05
T cell infiltration into tumor islets (/0.93 mm^2)	
0	7
+: 1–20	22
++: ≧20	1
IL-12 responsiveness (No. of positive IL-12 stimulated IFN-γ producer[a] (total, $n = 30$))	
$CD4^+$ T cells	2
$CD8^+$ T cells	6
$CD4^+$ and/or $CD8^+$ T cells	7

[a] IL-12 responsiveness was regarded as positive when $CD4^+$ T and/or $CD8^+$ T cell populations from each patient produced more than 20 pg/ml of IFN-γ following stimulation with IL-12

Materials and methods

Subjects

A total of 30 newly diagnosed colon and rectum cancer patients who were scheduled for surgery were enrolled in this study at the Kinki University School of Medicine from July 2002 through August 2005. Among the 30 were five stage IV colon/rectum cancer patients without lung and liver metastasis who would benefit from surgery. Other stage IV patients who would not benefit from surgery were excluded. None of the patients had yet received any kind of treatment. They were characterized by their icGSH index and degree of TIL in tumor islets, and the IL-12 responsiveness of their CD4 and CD8 T cells (Table 1). The follow-up period after surgery ranged from 48 to 60 months. Patient characteristics are shown in Table 1.

Thirty-eight age-matched healthy subjects ranging from 40 to 80 years of age were recruited from individuals attending the Louis Pasteur Center for Medical Research for routine health checkups as controls. They had no history of cancer, chronic infectious or autoimmune diseases, diabetes mellitus, nephritis or asthma. In addition to the 38 healthy subjects above, the GSH index was determined for 12 patients with advanced colorectal cancer with multimetastasis, each of whom had received medical care at the polyclinic of the Louis Pasteur Center for Medical Research from April 2002 through August 2006. They had

a history of recurrence, and had already received different treatments, such as surgery, chemotherapy, radiotherapy and/or immunotherapy.

One colon cancer and eight breast cancer patients, who were going to receive adoptive immunotherapy at the Kan Clinic before and after OK-432 administration, were also assessed for their GSH index and Mo T cell stimulatory activity. For in vitro testing and an HPLC assay, we used Mo obtained from cancer-bearing patients at the Louis Pasteur Center for Medical Research who were undergoing immunotherapy using the leukopheresis method.

Blood samples were taken from all cancer and healthy subjects after obtaining informed consent; these samples were always used within 8 h.

Cytokines and reagents

Recombinant human IL-12 (rIL-12) was provided by the Genetics Institute (Cambridge, MA), anti-$CD56^-$, $CD14^-$, $CD8^-$ and CD4-conjugated MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) were used for Mo and $CD4^+$ T cell separation. For T cell detection in tumor cell islets, formalin-fixed and paraffin-embedded sections were stained with an anti-CD45RO antibody (UCHL1, IgG2a, Nichirei Corp., Tokyo) according to the manufacturer's recommended procedures.

Preparation of peripheral blood lymphocyte and monocyte populations

Venous blood was taken from both cancer bearing and healthy subjects and the peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque gradient centrifugation method. $CD56^+$ NK cells, $CD14^+$ Mo, $CD8^+$ T cells and $CD4^+$ T cells were positively separated using Midi-MACS LS^+ columns. The separated $CD14^+$ cells were used for icGSH staining and $CD8^+$ and $CD4^+$ T cells to determine IL-12 responsiveness, as described below. $CD4^+$ T cells from a healthy person were used to determine the T cell stimulatory capacity of the Mo.

GSH staining and determination of the GSH index

The GSH index was determined 2 or 3 days before surgery. To determine Mo icGSH levels, PBMC and isolated $CD14^+$ Mo were allowed to adhere to glass slides in a culture medium for 30 min then washed, and stained with 250 mM monochlorobimane (MCB, Molecular Probes, Inc. OR, USA) in 10% FBS-PBS for 15 min. Cells were then fixed with 0.72% formalin (TAAB Lab, England) in 10% FBS-PBS and after 10 min, they were embedded in Aqua Poly/Mount (Polysciences, Inc, PA). Cells were monitored by fluorescence microscopy, and images were acquired with a Hamamatsu C5810 3CCD camera using Sicon image software under an Olympus BX50WI lens using a WV filter. After converting Mo to a gray image, their icGSH levels were measured with an Image J 1.32 software that examined the area and mean intensity of each Mo. The formula GSH index = average (area × mean intensity) was used to determine the GSH index.

HPLC assay for icGSH in Mo

As 1–2 × 10^6 Mo are required to measure the amount of GSH by HPLC, we obtained Mo from ten patients who were undergoing immunotherapy at the Louis Pasteur Center for Medical Research using the leukopheresis method ($n = 6$, digestive cancer patients; $n = 2$, breast cancer; $n = 2$, others). As shown in Supplement Fig. 1, a positive correlation between the GSH index and the amount of reductive icGSH was found ($R = 0.78$, $P = 0.0044$). To validate the GSH index, $CD14^+$ Mo from the above ten cancer subjects were tested for both the GSH index by MCB staining, and the quantity of GSH by high performance chromatography (HPLC) with a gold electrode as described previously [8]. These methods determine GSH and are not affected by GSSG. However, as only 1–2 × 10^3 Mo are required to determine the icGSH index by staining, these results suggest that the amount of icGSH in Mo is indicative of the GSH index, and is useful in measuring an individual patient's icGSH.

Immunostaining of $CD45RO^+$ T cells

TIL levels were determined using paraffin-embedded pathological specimens from the resected tumor. T cells were stained with anti-CD45RO Ab using formalin-fixed, paraffin-embedded sections as recommended by the manufacturer. The tumor mass was determined in a given visual field at a magnification of ×200 (0.933 mm^2). Infiltrating effecter/memory $CD45RO^+$ T cells were counted in the tumor cell islets [9].

Determining IL-12 responsiveness

The IL-12 responsiveness of preoperative patients was determined using positively isolated $CD4^+$ and $CD8^+$ T cells from each patient which were cultured with 1,000 pg/ml rIL-12 for 20 h. Supernatants were extracted and the concentration of IFN-γ was quantified using enzyme-linked immunosorbent assay (ELISA) as previously described [10].

Determination of Mo T cell stimulating activity

The GSH index and Mo T cell stimulating activity were simultaneously determined in 20 tumor-bearing patients; the latter by a modified method of Romani et al. [11]. 10^5/well Mo from each patient and 2×10^5/200 μl/well $CD4^+$ T cells from healthy subjects were mixed together with 10 ng/ml anti-CD3 mAb (Orthoclone OKTR3Inj, Ortho Pharmaceutical Corp., USA) and cultured for 20 h in TC MICROWELL 96F plates (Nunc A/S Denmark). $CD4^+$ T cells from the same healthy subject were aliquoted and stored in liquid nitrogen, so that the same standardized cells could be used for every experiment. Supernatants were extracted and IFN-γ was quantified by ELISA. Anti-CD3 antibodies presented by Mo via Fc receptors were stimulated via the T cell receptors of $CD4^+$ T cells. This experimental system thus models Mo–T cell interactions, making it possible to evaluate the antigen-presenting activities of Mo without MHC restriction.

Statistical analysis

All values in the text and tables are presented as mean ± SEM. Statistical analysis of the data was performed using the Student's t test. The relationships between GSH index and the presence of $CD45RO^+$T cells in tumor islets were estimated using logistic regression analysis. Cox proportional hazards regression models and Kaplan–Meier analysis were used to investigate whether or not the GSH index and/or clinical prognostic factors were related. Calculations were performed using the JMP 6.0.3 statistical software package (SAS Institute Inc, Cary, NC).

Results

GSH index in patients with colorectal cancer and healthy subjects

The GSH indices were determined for the 30 preoperative colorectal cancer patients and 38 healthy controls were determined. As shown in the comparative line histogram (Supplement Fig. 2), healthy subjects exhibited a normal probability distribution for the Mo-GSH index, peaking between values of 0.7 and 0.9, whereas patients with colorectal cancer also exhibited a normal probability distribution but with a slightly higher peak value of 0.9–1.1. The mean values of GSH indices for healthy subjects and colorectal cancer patients were found to be 0.86 ± 0.04, and 0.92 ± 0.05, respectively. In addition, no correlation was found between GSH index and cancer stages in operable patients (stage 0, I: 0.79 ± 0.20; II: 0.89 ± 0.09; III: 0.86 ± 0.08; IV: 1.19 ± 0.13) (Table 1). This would explain why the stage IV patients in this study had high GSH index, and 12 other patients with recurring colorectal cancer in the advanced stage were distributed on both ends of the GSH index with two peaks at 0.5–0.7 and 1.1–1.3 GSH index (Supplement Fig. 2). These results demonstrate that there is no significant correlation between GSH index and cancer stage; however, it was noted that GSH index tend to decrease in the terminal stage of cancer.

Correlations between the Mo icGSH index and TIL into tumor islets

Many previous studies have explored the relationship between TIL in tumor islets and prognosis for tumor-bearing patients; however, almost no studies on the relationship of TIL in the local tumor site and Mo icGSH [9, 12] exists, therefore we felt the need to perform the present study.

Logistic regression analysis of the Mo-GSH index and presence of TIL in tumor islets in 30 preoperative patients revealed a positive association ($P < 0.001$). The 50% cut-off value for the GSH index positively correlated with the presence of TIL and was nearly 0.7. Thus, patients were divided into two clear subgroups determined by the presence or absence of TIL within tumor cell islets and corresponding to their GSH indices; GSH indices of ≥0.7 corresponded closely with the TIL presence in tumor islets group ($n = 23$, 1.03 ± 0.04), and GSH indices of <0.7 corresponded with the TIL absence in tumor islets group ($n = 7$, 0.55 ± 0.08, $P < 0.001$; Fig. 1a). Our previous results suggest that Mo with high icGSH and low icGSH represent two functionally distinct Mo subsets that may exert anti- and pro-tumor functions, respectively [5]. Therefore, we designated Mo with a GSH index ≥0.7 as *reductive* (R)-Mo, and those with an index of <0.7 as *oxidative* (O)-Mo. Figure 1b shows representative examples of MCB staining in two colon cancer patients with R-Mo or O-Mo. As shown in Fig. 1a, these results demonstrate that there is a positive correlation between higher Mo icGSH and positive TIL into the tumor islets.

GSH index and TIL in tumor islets correlate with clinical outcome

It is well documented that the presence of TIL correlates with improved survival in patients with colorectal cancer [3, 12]. Therefore, our results showing that R-Mo is associated with the presence of TIL prompted us to examine the possibility that R-Mo may correlate with improved survival of colorectal cancer patients. The GSH index (≥0.7 or <0.7) and the presence or absence of TIL in tumor islets as predictor variables were analyzed in the group of 30 patients (stages 0–IV) using Cox's proportional hazards

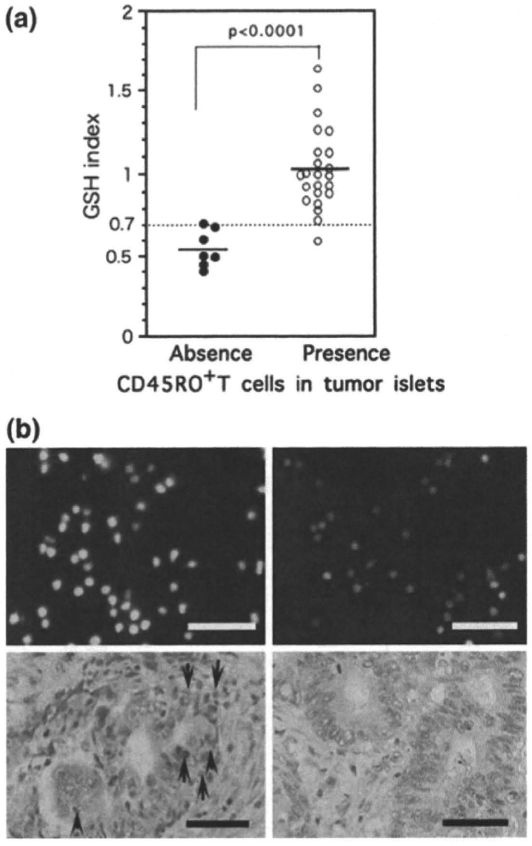

Fig. 1 **a** Comparison of GSH index and TIL in tumor islets. The mean value of the GSH index in patients whose tumor cell islets were infiltrated with TIL was significantly higher than in patients without TIL (absence of TIL, 0.561 ± 0.052 vs. presence of TIL, 1.035 ± 0.051). **b** Comparison of the icGSH index of Mo and TIL in tumor islets of two patients with colon cancer (*left panel* 53 years old, female, stage II; *right panel* 62 years old, female, stage II). *Bar* 50 μm. *Upper panels* MCB staining of the Mo (GSH index of *left panel*: 1.07 vs. *right panel*: 0.41). *Lower panels* Histological section of $CD45RO^+$ T cells in tumor islets (*left panel*: several TIL in tumor islets ↑). On the right-hand side there is no TIL into the tumor islets

regression models with overall survival as the dependent variable. The same was done separately for a group of 23 stage II and III patients with moderately advance tumor. Although multivariate analysis did not show significant results in terms of a strong correlation, univariate analysis showed that the GSH index and TIL presence/absence were significant independent factors that could predict survival in both groups of patients (Table 2).

Using the Kaplan–Meier model, the overall survival outcome of stage 0–IV cancer patients was analyzed with respect to (1) Mo redox status comparing R-Mo and O-Mo, (2) the presence or absence of TIL in tumor cell islets, and (3) with respect to cancer stage. A significant difference was found in the overall survival rates of the 30 preoperative patients (Supplement Fig. 3A) and 23 stage II and III patients (Fig. 2a) with R-Mo or O-Mo in the log-rank test. The 4-year mortality rate of stage II and III patients (all preoperative patients) with R-Mo was found to be 6.2% (13%), whereas O-Mo was 66.6% (57.1%), demonstrating that R-Mo patients have a superior survival rate. Consistent with previous studies [9, 12], there was also a significant difference between patients with and without TIL in tumor islets in the log-rank test (Fig. 2b; Supplement Fig. 3B). On the other hand, no correlation was found between tumor stage and overall survival (Supplement Fig. 3C). Furthermore, no significant difference was found in disease-free survival of stage II and III patients between the R-Mo/O-Mo ($P = 0.205$) group and TIL existence/absence ($P = 0.054$) group. However, R-Mo and TIL existence patients showed better outcomes than the O-Mo and TIL absence groups.

These data indicate that knowing R-Mo or O-Mo status is as equally predictive of patient survival as knowing whether TIL is present or absent in tumor islets. Both reveal the same kind of information about local anti-tumor response, and result in almost the same predictive value. Therefore, even without using resected specimens to know if TIL is present or not, we are still able to generate the same conclusion using R-Mo or O-Mo status.

Relationships between GSH indices and $CD4^+$ and/or $CD8^+$ T cell responsiveness to IL-12

The evidence from histology and survival of colorectal patients described above suggests that anti-tumor responses occur at high frequency in patients with R-Mo. In a previous paper, we demonstrated that IL-12 responsiveness was a good indicator that allowed us to predict the existence of sensitized T cells as part of an ongoing anti-tumor immune response [10]. Based on these findings, we compared IL-12 responsiveness among patients with colorectal cancer as a parameter for the occurrence of anti-tumor response. Seven of 30 patients showed IL-12 responsiveness of $CD4^+$ T and/or $CD8^+$ T cells (Table 1, $n = 30$). IL-12 responsiveness of T cells was identified as an independent factor significant in predicting survival using Cox proportional hazards regression analysis (Table 2, $P = 0.04$). Although there was no statistically significant correlation between patients with or without IL-12 responsiveness in the Kaplan–Meier analysis ($P = 0.11$), positive IL-12 responsiveness ($n = 7$) was only found in patients with moderate GSH indices of R-Mo (supplement Fig. 3D). Furthermore, it is noteworthy to point out that all patients who showed IL-12 responsiveness lived for more than 5 years, including two stage IV patients, despite their advanced status. When comparing R-Mo colorectal cancer patients with those showing TIL presence and those with IL-12 responsiveness, the relationship is such that the R-Mo group and TIL presence group mostly overlap but

Table 2 Prognostic factors in patients with colorectal cancer using Cox proportional hazard model

Variables	Categories	Univariate analysis	
		Overall survival	
		HR (95% CI)	*P*
Stage	Stage 0: I ($n = 2$) versus II ($n = 9$) versus III ($n = 13$) versus IV ($n = 5$)	2.27 (0.85–6.85)	0.103
Stage II, III	Stage II ($n = 9$) versus III ($n = 13$)	2.71 (0.40–53.05)	0.33
GSH index (stage I, II, III, IV; $n = 30$)	≥ 0.7($n = 23$) versus <0.7($n = 7$)	0.194 (0.038–0.887)	0.0352*
GSH index (stage II, III; $n = 23$)	≥ 0.7($n = 17$) versus <0.7($n = 6$)	0.061(0.003–0.421)	0.004**
TIL in tumor islets (stage I, II, III, IV; $n = 30$)	Presence ($n = 23$) versus absence ($n = 7$)	0.194 (0.038–0.887)	0.0352*
TIL in tumor islets (stage II, III; $n = 23$)	Presence ($n = 16$) versus absence ($n = 7$)	0.083 (0.0042–0.565)	0.01**
IL-12 responsiveness ($n = 30$)	Positive (CD4 T and/or CD8 T) ($n = 7$) versus negative ($n = 23$)	1.86e−7 (– –0.898)	0.04*
IL-12 responsiveness (stage II, III; $n = 23$)	Positive (CD4 T and/or CD8 T) ($n = 5$) versus negative ($n = 18$)	5.9e−7 (– –2.03)	0.150

* $P < 0.05$, ** $P < 0.01$

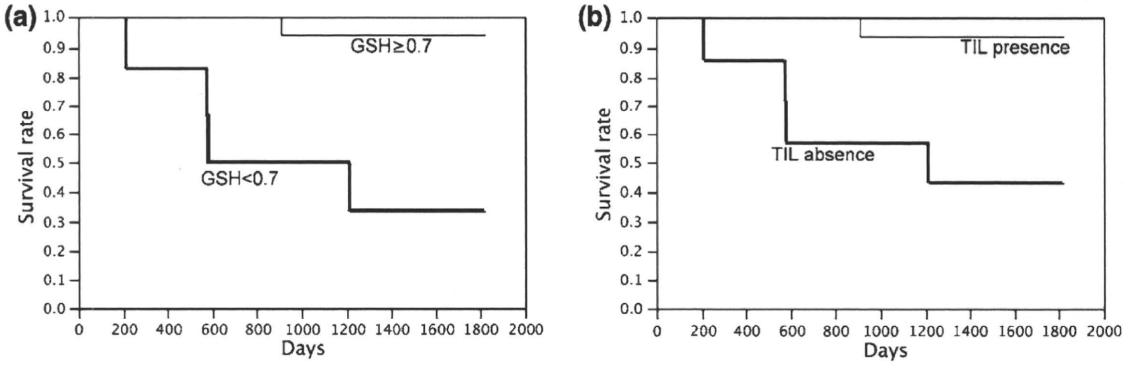

Fig. 2 **a** Kaplan–Meier analyses of stage II and III colorectal cancer patients ($n = 23$): patients with R-Mo (GSH $\geq$ 0.7) or O-Mo (GSH < 0.7) plotted separately ($P = 0.0009$). **b** Kaplan–Meier analyses of stage II and III colorectal cancer patients ($n = 23$): patients with or without intratumoral T cells plotted separately ($P = 0.0048$)

not completely, whereas the IL-12 responsiveness group is contained within the range of both groups (Supplement Fig. 3D).

GSH index and T cell stimulatory activity of Mo

The results presented here suggest that anti-tumor immune responses work more efficiently in patients with R-Mo. This led us to explore whether R-Mo would induce stronger $CD4^+$ T cell stimulatory activity than O-Mo. To evaluate the antigen-presenting capacity of Mo distinct in their intracellular redox status, we used an assay system using anti-CD3 antibodies presented by Mo via Fc receptors. This experimental model of Mo–T cell interaction makes it possible to evaluate the antigen-presenting activities of Mo without MHC restriction. As shown in Fig. 3a, R-Mo stimulated $CD4^+$ T cells more strongly than O-Mo (evaluated as IFN-γ production). These results indicate that R-Mo with a higher icGSH can stimulate T cells more effectively than O-Mo and induce Th1 responses.

Hamuro et al. showed that it is possible to change the GSH status of Mo from oxidative to reductive in vivo and in vitro [6, 13], and the functional plasticity of Mo has been demonstrated elsewhere [14, 15]. This led us to question whether treatments to increase the GSH index would affect the $CD4^+$ T cell stimulatory activity of Mo. Several kinds of biological response modifiers (BRM) have been shown to increase monocyte GSH levels. However, for our purposes, we selected a Streptococcal preparation of OK-432 commonly used in Japan as the BRM agent, and it allowed the GSH indices of Mo and T cell stimulation activities to be evaluated prior to and following treatment [16]. As shown in Figs. 3b-1 and b-2, GSH indices of Mo significantly increased, while their $CD4^+$ T cell stimulatory

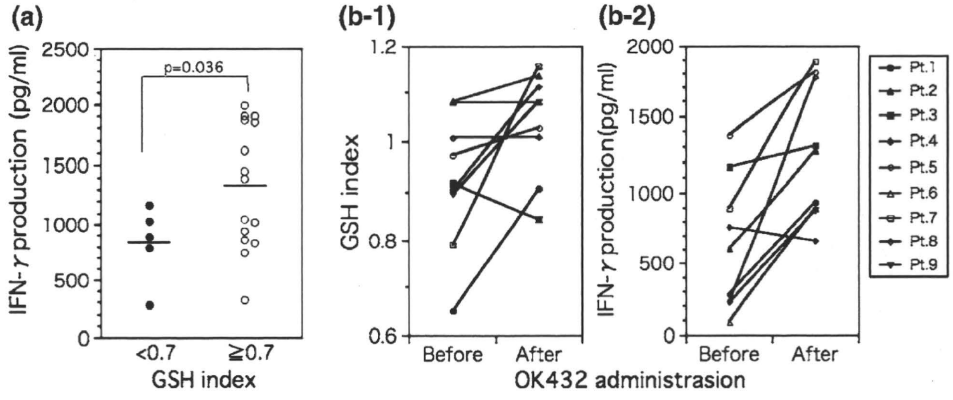

Fig. 3 **a** Comparison of T cell stimulatory activity of O-Mo and R-Mo in vitro. R-Mo (GSH $\geq$ 0.7) stimulate T cells more strongly compared with O-Mo (GSH $<$ 0.7) (O-Mo; 829.0 $\pm$ 152.0 vs. R-Mo; 1314.3 $\pm$ 136.4 pg/ml, $P = 0.036$). **b** Effect of OK-432 administration on **a** the GSH index and **b** T cell stimulatory activity of Mo in vitro. After OK-432 administration, GSH indices increased dramatically compared to pre-therapy (**b-1**) (before: 0.923 $\pm$ 0.046 vs. after: 1.039 $\pm$ 0.035, $P = 0.041$). T cell stimulatory activity of Mo increased after OK-432 treatment (**b-2**) (before: 626.8 $\pm$ 152.8 vs. after: 1271.9 $\pm$ 153.7 pg/ml, $P = 0.003$)

activities measured as IFN-γ production also increased. These findings suggest that medical intervention using an agent to change the intracellular GSH redox status of Mo from an oxidative status to a reductive one would increase $CD4^+$ T cell stimulatory activities and thus successfully induce Th1 responses as shown by higher IFN-γ production.

Discussion

We have demonstrated that patients bearing colorectal tumors with R-Mo, containing elevated intracellular levels reductive GSH, have correlated extended lymphocyte infiltration into the tumor islets (Fig. 1a, b) and exhibit better overall survival [9] (Fig. 2a, b; Table 2; supplement Fig. 3a, b). R-Mo stimulates T cells more efficiently than O-Mo (Fig. 3a), and as a result, IL-12 responsiveness was found only in patients with R-Mo (Supplement Fig. 3D). The presence of TIL in the tumor islets combined with a high incidence of IL-12 responsiveness suggests that anti-tumor Th1 immune responses may have been induced and was ongoing in these patients with high intracellular GSH. Several previous research have shown that the presence of TIL in tumor islets is a good prognostic biomarker for patients with colorectal cancer [4, 9, 12], and that GSH levels in antigen-presenting cells have an effect on Th1 response. However, this is the first report to demonstrate that R-Mo with a higher icGSH facilitates the induction of anti-tumor immune responses (presumably Th1), and leads to better overall survival in R-Mo patients. These results demonstrate a significant advantage in using GSH index for stage classification for predicting overall survival.

Our ultimate purpose in pursuing this research is to improve the survival rate of solid turmor patients undergoing anti-tumor therapy, and to identify appropriate biomarker parameters for monitoring anti-tumor immunity using easily obtainable peripheral blood specimens. Functional immunity can be monitored in vitro using peripheral blood with tests such as natural killer cell activity [17], IFN-α producing ability [18], and proliferative responses or cytokine production by mitogenic stimulation. However, these tests do not necessarily reflect anti-tumor T cell responses in local tumor sites. Although it is possible to determine antigen-specific responses through IFN-γ secretion or tetramer assays, they are difficult to apply to undefined antigens. In situ analysis of tumor-infiltrating immune cells in resected colorectal tumors has been demonstrated to be a valuable prognostic tool [3, 12]. The clinical significance of determining this information through peripheral blood specimen alone, before or without surgery, has marked advantages for optimizing and individualizing therapies. Establishing the Mo GSH index requires only 3 ml of peripheral blood; therefore, it is suitable for routine clinical use.

Why do icGSH levels relate to anti-tumor activity? There are several reports that activated Mf/Mo icGSH levels are higher than control Mf/Mo [19, 20]. Furthermore, the capacity of allo-stimulatory and IFN-γ production correlated with icGSH levels in Mo-derived dendritic cells [21]. Low intracellular GSH levels in antigen-presenting cells correlated with defective processing of antigens, indicating that this thiol may be a critical factor in regulating antigen-processing [22]. Furthermore, GSH and IL-2 are involved in the growth and replication of activated lymphocytes [23]. These results suggest that the icGSH levels of Mo/Mf/DC affect not only anti-tumor activity, but also the antigen-presenting activities of Mo/DC. As our data that will appear in a follow-up report will suggest that there is a difference in the plasma cytokine/chemokine

levels in the tumor environment in R-Mo and O-Mo patients (analysis is now ongoing; data not shown). Additionally, it is easy to anticipate that the icGSH of Mo influences lymphocyte proliferation, differentiation, movement and chemokine/cytokine production [24, 25].

Recently, analogous to the Th1/Th2 nomenclature, the concept of polarized M1 and M2 macrophages has emerged and is commonly used [15, 26]. M1 cells are characterized as (a) having a high IL-12 and low IL-10 producer phenotype, (b) being proficient generators of effecter molecules such as reactive oxygen, nitrogen intermediates and inflammatory cytokines, and (c) contributing as inducer and effecter cells of polarized Th1 responses. On the other hand, the various forms of M2-Mf share a low IL-12 producer phenotype, and have a tendency to shift from arginine metabolism to production of ornithine and polyamines via arginase [2]. Our present results suggest that R-Mf closely correspond to M1-Mf and O-Mf to M2-Mf [5].

Mantovani et al. [1] demonstrated that Mf are versatile cells that can express different functional programs in response to micro-environmental signals. Similarly, Hamuro et al. demonstrated that the skewing toward Th1-biased responses induced by lentinan, a well-known BRM, was mediated via distinctive Mo cytokine production patterns with elevated intracellular GSH content in vitro [27] and in vivo [28]. Our results with Mo of OK-432-treated patients support the notion that increased Mo icGSH levels skew responses toward the Th1-type, and result in an improved outcome for tumor-bearing patients. Recently, a school of thought has emerged that tumor-associated Mo/Mf are good targets for therapy because they play a key role as regulators in the development of the tumor micro-environment and impact on resulting anti-tumor immune responses. We strongly support the idea that the status of Mo can be appropriately used as a biomarker for prognostic purposes. Our present results indicate that Mo status does indeed influence the tumor-micro-environment and anti-tumor immune responses, and that their plasticity indicates that they are potential targets for novel therapeutic approaches [29, 30]. Alteration of the Mo/Mf redox status will make it easier and practical to induce anti-tumor activity.

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References

1. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 23:549–555
2. Mantovani A, Sozzani S, Locati M, Schioppa T, Saccani A et al (2004) Infiltration of tumours by macrophages and dendritic cells: tumour-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Novartis Found Symp 256: 137–145 (Discussion 146–138, 259–169)
3. Galon J, Fridman WH, Pages F (2007) The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 67:1883–1886
4. Pages F, Galon J, Fridman WH (2008) The essential role of the in situ immune reaction in human colorectal cancer. J Leukoc Biol 84:981–987
5. Murata Y, Shimamura T, Hamuro J (2002) The polarization of T(h)1/T(h)2 balance is dependent on the intracellular thiol redox status of macrophages due to the distinctive cytokine production. Int Immunol 14:201–212
6. Dobashi K, Aihara M, Araki T, Shimizu Y, Utsugi M et al (2001) Regulation of LPS induced IL-12 production by IFN-gamma and IL-4 through intracellular glutathione status in human alveolar macrophages. Clin Exp Immunol 124:290–296
7. Peterson JD, Herzenberg LA, Vasquez K, Waltenbaugh C (1998) Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci USA 95:3071–3076
8. Tada-Oikawa S, Kato T, Kuribayashi K, Nishino K, Murata M et al (2008) Critical role of hydrogen peroxide in the differential susceptibility of Th1 and Th2 cells to tributyltin-induced apoptosis. Biochem Pharmacol 75:552–561
9. Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K et al (1998) CD8 + T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 58:3491–3494
10. Uno K, Mitsuishi Y, Tanigawa M, Okuno K, Hirai N et al (2003) A series of immune responses leading to the induction of T cell IL-12/IL-18 responsiveness in patients with relatively large tumor burdens. Cancer Immunol Immunother 52:33–40
11. Romani N, Inaba K, Pure E, Crowley M, Witmer-Pack M et al (1989) A small number of anti-CD3 molecules on dendritic cells stimulate DNA synthesis in mouse T lymphocytes. J Exp Med 169:1153–1168
12. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B et al (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313:1960–1964
13. Murata Y, Amao M, Hamuro J (2003) Sequential conversion of the redox status of macrophages dictates the pathological progression of autoimmune diabetes. Eur J Immunol 33:1001–1011
14. Biswas SK, Sica A, Lewis CE (2008) Plasticity of macrophage function during tumor progression: regulation by distinct molecular mechanisms. J Immunol 180:2011–2017
15. Martinez FO, Gordon S, Locati M, Mantovani A (2006) Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol 177:7303–7311
16. Kan N, Kodama H, Hori T, Takenaka A, Yasumura T et al (1993) Intrapleural adaptive immunotherapy for breast cancer patients with cytologically-confirmed malignant pleural effusions: an analysis of 67 patients in Kyoto and Shiga Prefecture, Japan. Breast Cancer Res Treat 27:203–210
17. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K (2000) Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356:1795–1799
18. Uno K, Hirosaki M, Kakimi K, Tominaga M, Suginoshita Y et al (2007) Impaired IFN-alpha production and the risk of cancer development. J Interferon Cytokine Res 27:1013–1017

19. Sarkar K, Bose A, Chakraborty K, Haque E, Ghosh D et al (2008) Neem leaf glycoprotein helps to generate carcinoembryonic antigen specific anti-tumor immune responses utilizing macrophage-mediated antigen presentation. Vaccine 26:4352–4362
20. Klimberg VS, Kornbluth J, Cao Y, Dang A, Blossom S et al (1996) Glutamine suppresses PGE2 synthesis and breast cancer growth. J Surg Res 63:293–297
21. Kuppner MC, Scharner A, Milani V, Von Hesler C, Tschop KE et al (2003) Ifosfamide impairs the allostimulatory capacity of human dendritic cells by intracellular glutathione depletion. Blood 102:3668–3674
22. Lemaire G, Guittet O, Vesin MF, Lepoivre M, Cottet MH (2009) Glutathione depletion reveals impairment of antigen processing and inhibition of cathepsin activity by nitric oxide in antigen-presenting cells. Mol Immunol 46:1100–1108
23. Liang CM, Lee N, Cattell D, Liang SM (1989) Glutathione regulates interleukin-2 activity on cytotoxic T-cells. J Biol Chem 264:13519–13523
24. Desai A, Huang X, Warren JS (1999) Intracellular glutathione redox status modulates MCP-1 expression in pulmonary granulomatous vasculitis. Lab Invest 79:837–847
25. Hashimoto S, Gon Y, Matsumoto K, Takeshita I, MacHino T et al (2001) Intracellular glutathione regulates tumour necrosis factor-alpha-induced p38 MAP kinase activation and RANTES production by human bronchial epithelial cells. Clin Exp Allergy 31:144–151
26. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164:6166–6173
27. Murata Y, Shimamura T, Tagami T, Takatsuki F, Hamuro J (2002) The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. Int Immunopharmacol 2:673–689
28. Yamada J, Hamuro J, Hatanaka H, Hamabata K, Kinoshita S (2007) Alleviation of seasonal allergic symptoms with superfine beta-1, 3-glucan: a randomized study. J Allergy Clin Immunol 119:1119–1126
29. Allavena P, Sica A, Solinas G, Porta C, Mantovani A (2008) The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol 66:1–9
30. Sica A, Rubino L, Mancino A, Larghi P, Porta C et al (2007) Targeting tumour-associated macrophages. Expert Opin Ther Targets 11:1219–1229

特集 フローチャートでみる私の処方
III. 循環器疾患の処方

2．川崎病（心合併症を含む）

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KEY WORDS　冠動脈病変，免疫グロブリン，ステロイド，インフリキシマブ

はじめに

川崎病は小児期に好発する原因不明の血管炎症候群で，一部は治療に抵抗し，あるいは反応不良で冠動脈病変を生じる[1]。できる限り早期に血管炎を鎮静化させることが結果として生じる冠動脈病変の発症頻度を抑制することに寄与する。本稿では2003年に発表された急性期治療ガイドライン[2]（http://www.kawasaki-disease.org/tebiki/pdf/guide.pdf），おなじく2008年に発表された心臓血管後遺症の診断と治療に関するガイドライン[3]（http://www.j-circ.or.jp/guideline/pdf/JCS2008_ogawasy_d.pdf）を中心に最新の知見を交え，急性期から遠隔期までの治療方針に関してフローチャート（図）に沿って解説する。

Ⅰ．急性期の初期治療

現在最も信頼される治療法は免疫グロブリン超大量療法（IVIG）2 g/kg単回投与とアスピリン30mg/kg/dayの併用である。かつては分割投与が広く行われていたが近年はほとんどの症例で2 g/kgないし1 g/kgの超大量療法が行われるようになった[4]。

IVIG 開始は7病日以内に開始することが望ましく，特に冠動脈拡張病変が始まるとされる9病日以内に解熱することが重要である。そのため症状がそろわず診断に苦慮する不全型症例に対しても他の疾患が否定的であれば積極的に IVIG を投与する。IVIG を5病日未満に投与した症例で初期治療不応例が多いという全国調査結果[5]から早期診断例に対して IVIG 投与を待機すべきといった意見もある。しかし原著論文では IVIG 不応例の割合は多いものの冠動脈病変合併率に差がないことから一定の治療効果があるのではないかと推論されている。早期診断例は重症例が多いのではないかとも推測されている[6]。そのため筆者らの施設では診断後速やかに IVIG 投与を行う方針としている。

IVIG は有効で副反応は少ないものの，投与時は心不全の発症および心機能低下の増悪に十分留意し，投与速度が速過ぎないように

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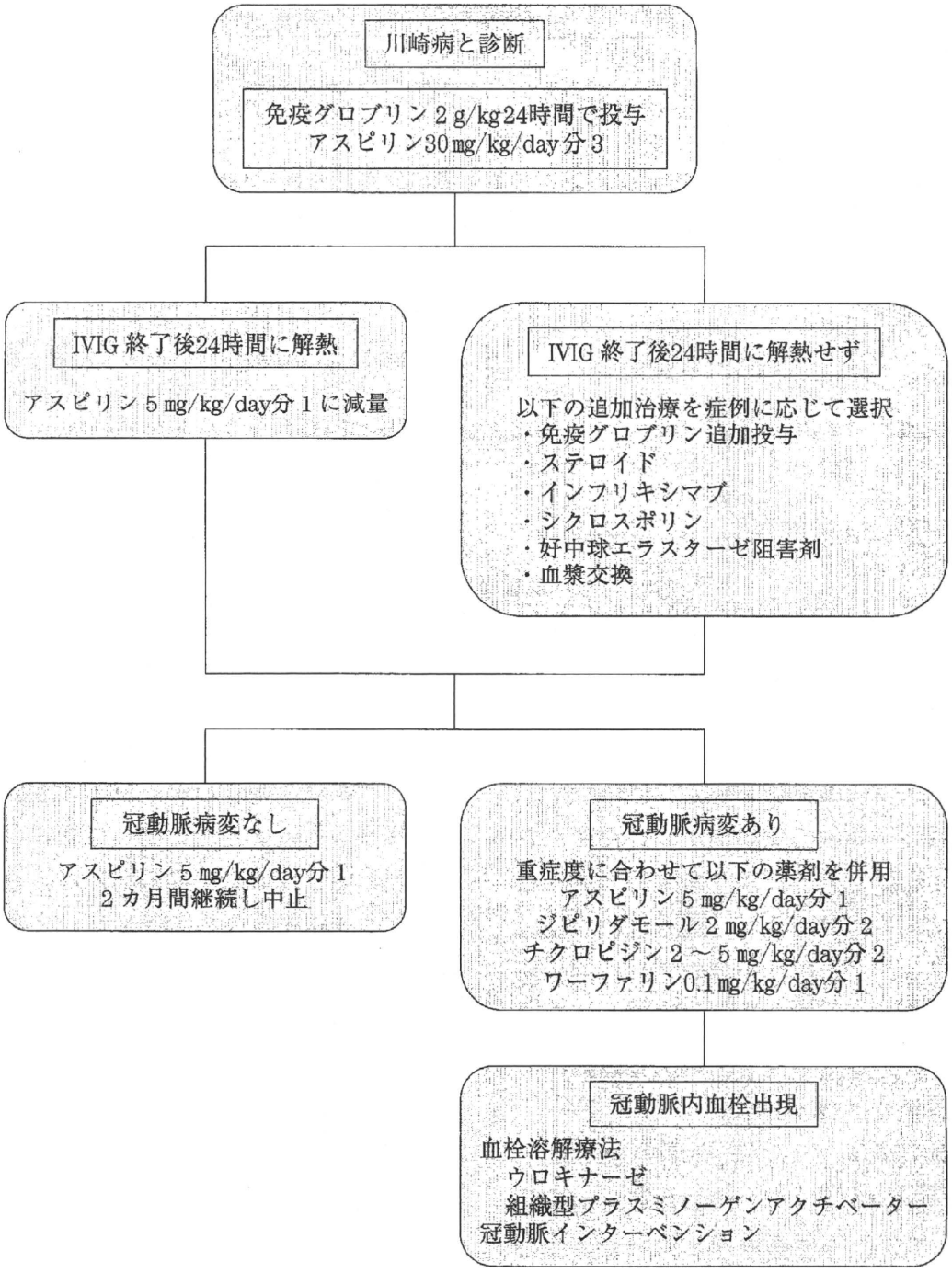

図　川崎病治療フローチャート

注意する。投与によるショック，アナフィラキシー様反応や，無菌性髄膜炎等の副反応に対しては十分な観察が必要である。

著者らは治療開始前に IVIG 反応性を予測して初期治療の層別化を行う新たな治療戦略を提唱し，現在全国規模の前方視的無作為化比較試験（RAISE Study：http://raise.umin.jp/）が行われている。

II．初期治療終了後の治療方針

1．IVIG 有効例に対する治療

IVIG 終了後24時間以内に解熱した症例は，アスピリンを５mg/kg/dayに減量する。数％に再燃を来す症例が存在するが，多くは再燃せずに経過し冠動脈病変も合併しない。

2．IVIG 不応例に対する治療

IVIG 終了後24時間において解熱効果がな