Hyper-IgM Syndrome Complicated with Interstitial Pneumonia and Peritonitis

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Hyper-IgM syndrome (HIM) is a rare disorder resulting from mutation in the CD40 ligand (CD40L) gene. This defect is associated with normal or elevated serum level of IgM and with low to undetectable levels of serum IgG, IgA, IgE. This case of HIM with CD40L deficiency was proven by flow cytometry but initially presented as interstitial pneumonia. *Pneumocystis carinii* pneumonia was highly suggested. After intravenous immunoglobulin and trimethoprim-sulfamethoxazole treatment, his lung condition improved. However, peritonitis developed and surgical intervention was performed. Ileum perforation and intestinal lymphoproliferation from a pathologic specimen were noted. Although peritonitis is extremely rare in patients with HIM, this report indicates that peritonitis which results from intestinal lymphoproliferation may be a manifestation of HIM. *(Chang Gung Med J 2003;26:53-9)*

Key words: **hypogammaglobulinemia, hyper-IgM syndrome, CD40 ligand, interstitial pneumonia, peritonitis.**

The syndrome of hypogammaglobulinemia with normal or increased IgM (HIM) is a rare disorder. It is illustrated by the failure of isotype switching in whom the CD 40 ligand (CD40L) gene is mutated. The CD40 is a surface antigen expressed on B cells. The CD40L is expressed on activated T cells.[1] These patients often develop opportunistic infections. The infections are usually of bacterial origin, and a unique predisposition to PCP has been well documented.[2,3] Peritonitis as the presenting manifestation of patients with HIM is extremely rare and no case has been reported in English literature (only one documented report in French.)[4] Here we report a case of HIM with CD40L deficiency that initially presented as interstitial pneumonia and developed peritonitis afterwards.

CASE REPORT

A previously healthy 2-year-old boy was admitted to our hospital because of a 10-day history of cough, rhinorrhea, and fever and a 2-day history of tachypnea. He had been taken to a local medical department for treatment but the symptoms persisted. Thus, he was brought to our pediatric emergency room where physical examination revealed an ill-looking boy with blood pressure of 104/60 mmHg, pulse rate of 90/min, respiratory rate of 46/min, body temperature of 36.8°C, and injected throat without exudate. He had no icteric sclera, anemic conjunctivae, or cervical lymphadenopathy. On auscultation, the chest had mild rales and rhonchi in both lung fields, and the heart beat was regular without obvi-

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ous murmurs. The abdomen was soft, flat and normoactive bowel sounds. The extremities were freely movable and the skin was normal. Complete blood counts revealed white blood cell (WBC) of 30620/mm^3, the differential count revealed segmented cells 2%, lymphocytes 43%, monocytes 7%, eosinophils 48%, red blood cells (RBC) 5.08×10^6/mm^3, hemoglobin 14.3 g/dl, hematocrit 41.7%, and platelet counts 564×10^3/mm^3. Therefore, he was admitted for further evaluation and treatment.

Results of routine serum biochemical examinations (including Na^+, K^+, Ca^{2+}, Glucose, BUN, Cr, GOT, and GPT) were all within reference limits. C-reactive protein (CRP) and erythrocyte sedimentation rate concentration were also within reference ranges. Blood culture, virus isolation (throat, rectum, and urine), urine routine, urine culture, stool routine and culture all revealed negative findings. Since leukocytosis with eosinophilia was noted, a series of studies were performed. The stool parasite study was negative. The IgM was 216 mg/dl (reference range, 43-207 mg/dl), IgG was 184 mg/dl (reference range, 345-1236 mg/dl), IgA was <6.7 mg/dl (reference range, 14-159 mg/dl), IgE was <10 IU/ml (reference range, 1.1-49 IU/ml), and total eosinophil count was 16390/mm^3 (reference range, 50-250/mm^3). Lymphocyte sub-populations included CD3 at 77% (65-85%), CD4 at 39% (35-55%), CD8 at 34% (20-34%), CD19 at 13% (5-15%), and CD4/CD8 ratio = 1.2 (1.0-2.1). C3, C4 and anti-dsDNA were within reference ranges. Chest radiography (CXR) showed increased infiltration with perihilar haziness over bilateral lung fields (Fig. 1A). Bone marrow aspiration showed eosinophilia myelo-

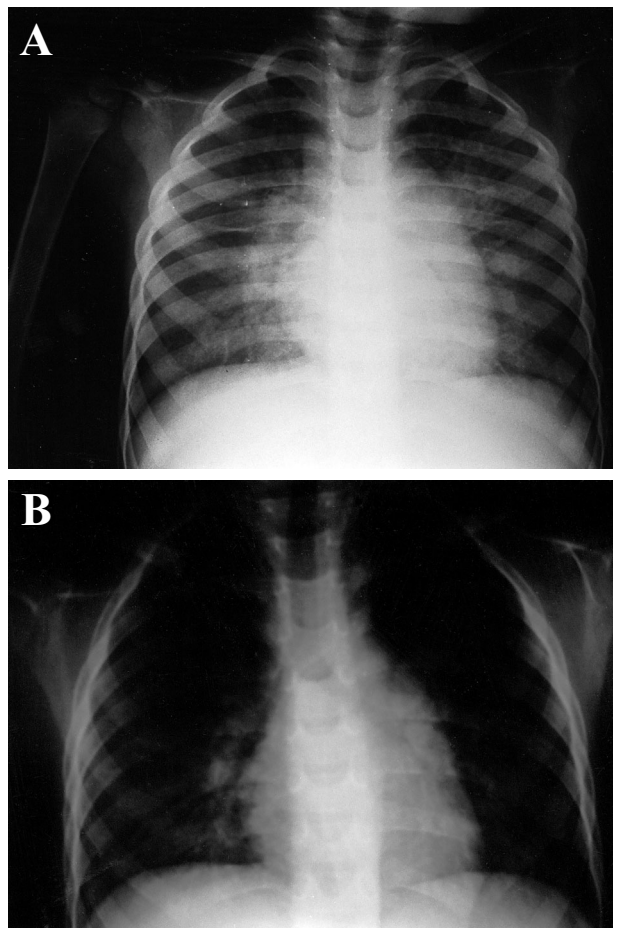

Fig. 1 (A) Chest X-ray shows increased infiltration with perihilar haziness over bilateral lung fields. (B) After treatment with IVIG and 5 days of TMP-SMZ therapy, the chest plain film reveals resolution of the interstitial pattern.

Table 1. CD40 Ligand (CD40L) Study by Flow Cytometry in HIM

	Patient	Patient	Age match control subject	Age match control subject
CD40L/CD4	(resting)	(PMA+A23187[a])	(resting)	(PMA+A23187[a])
(%)	5.70	6.80	12.40	58.30
CD40L/CD61	Patient	Patient	Age match control	Age match control
	(resting)	(thrombin[b])	(resting)	(thrombin[b])
(MFI)	22.4	28.7	42.7	120.3

[a] Percentage of CD40L expression : whole blood from patient and age-match control group were stimulated with PMA (32nM) and calcium ionophore A23187 (1g/ml) in the CD40L/CD4 study

[b] Mean fluorescence intensity of CD40L expression: whole blood from patient and age-match control group were stimulated with thrombin (0.5/ml) in the CD40L/CD61 study

Abbreviations: PMA: phorbol myristate acetate; MFI: mean fluorescence intensity; HIM: hyper-IgM syndrome

sis without young cells. HIM was highly suggested. CD40L was assessed using flow cytometry and CD40L deficiency was proven thereby (Table 1 and Fig 2). IVIG (Immune Serum Globulin, Pasteur Merieux, France; 400 mg/kg) was given. Based on clinical symptoms and signs, Sevatrin (intravenous form of trimethoprim-sulfamethoxazole (TMP-SMZ), 20mg TMP, 100 mg SMZ/kg/day) was tried under the impression of *pneumocystic carinii* pneumonia (PCP). Although his family refused further invasive evaluation for *P. carinii* (such as bronchoalveolar lavage or lung biopsy) and PCP could not be definitely proven, the clinical condition and CXR seemed to improve (Fig. 1B). However, fever developed again on the 23rd day after admission. Nausea and vomiting with abdominal pain were also noted. Physical examination of the abdomen showed diffuse tenderness, tympanic and hypoactive bowel sounds with muscle guarding and rebounding pain. Complete blood counts revealed WBC of 7400/mm^3, RBC at 4.39×10^6/mm^3, hemoglobin 11.4 g/dl, hematocrit 35.5%, and platelet counts 189×10^3/mm^3 and he differential count revealed band cells 18%, segmented cells 41%, lymphocytes 33%, monocytes 5%, eosinophils at 3%. CRP concentration was 23.6 mg/dl (reference range <0.6 mg/dl). Computed tomography (CT) of the abdomen showed extraluminal fluid and free air accumulation in the abdominal cavity (Fig 3). Peritonitis and hallow organ perforation were impressed. Emergent surgical intervention was performed and ileum perforation was noted. The pathological findings of the surgical specimen from the ileum resection showed mucosa necrosis, submucosa vascular congestion, hemorrhage and lymphocyte infiltration without evidence of malignancy. After surgery and antibiotic treatment, the

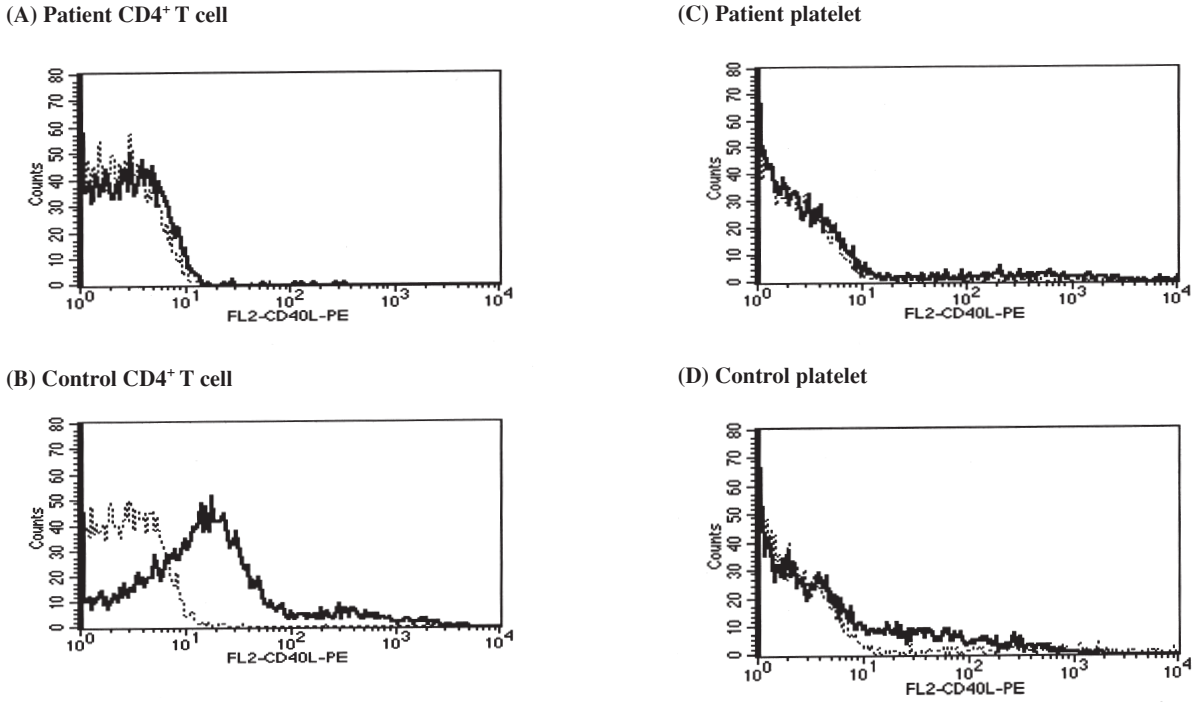

Fig. 2 Activated hyper-IgM patient T cells and platelets do not express CD40L. Peripheral blood T cells and platelets were purified from hyper-IgM patients ((A) and (C)) and healthy control subjects ((B) and (D)), which were incubated for 4 hours and 15 min in the presence or absence of PMA (32 nM) + A23187 (1ug/ml) for CD4 T cell and thrombin (0.5u/ml) for platelets, respectively. For both CD40L expression on CD4 T cells and platelets, the staining of cells stimulated with PMA (32 nM) + A23187 (1 ug/ml) or thrombin (0.5 u/ml) are shown by a solid line and the staining of unstimulated cells are shown by a dashed line. Stimulated cells from both patients and control subjects show no detectable binding of isotype-matched antibody used as specificity controls.

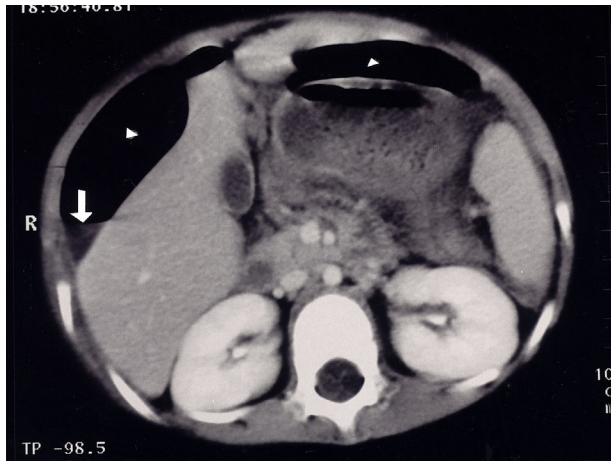

Fig. 3 Abdominal CT shows extraluminal fluid (arrow) and free air (triangle).

patient was discharged and regularly followed up at our department. Prophylatic antibiotics (Chemitrim; oral form of TMP-SMZ, 5 mg TMP, 25 mg SMZ/kg orally once daily) and IVIG infusion (400 mg/kg per 21 days) were also given. He remained healthy during 1 year of follow-up.

DISCUSSION

Immunodeficiency with hyper-IgM is a rare disorder, which was first described in 1961,[5] and is characterized by recurrent infections and very low levels of IgG, IgA, IgE with normal or elevated IgM.[3] Both primary and acquired forms of the disease have been reported. Among those with primary HIM, inheritance is usually X linked.[6] Acquired HIM may be secondary to congenital rubella,[7] neoplasia[8] or use of anti-epileptic drugs.[9]

Laboratory findings of this patient have shown that all forms of HIM are characterized by markedly reduced serum IgG, and a normal number of circulation B cells.[3] The molecular basis for X-linked HIM has been established as impaired function of CD40L.[10] The gene of CD40L is located at Xq26[11] and CD40- CD40L interaction are critical for T-cell dependent isotype switching. The gene Xq26 product, CD154, is the ligand for CD40 on B cells; it is upregulated on activated T cells as well as platelets.[12] Mutation in CD154 on activated T cells results in an inability to signal B cells to undergo isotype switching, and thus they produce only IgM. In addition to genetic analysis, HIM can also be diagnosed by demonstration of the absence of CD40L on activated T cells or platelets using flow cytometry.[13-15] The expression of CD40L on activated platelets using flow cytometry is also useful as a neonatal screening tool for X-linked HIM.[15]

We evaluated the CD40L expression on $CD4^+$-T cells and platelets in this patient as previous prescribed. The patient's peripheral venous blood was drawn into sterile tubes containing heparin (Becton Dickinson). Within 1 hour, 200 μL of whole blood was mixed with 20 μL of appropriate monoclonal antibody conjugates for 30 minutes (4°C in darkness). The following antibodies were used for staining: anti-CD3, anti-CD4, anti-CD61 phycoerythrin (Becton Dickinson) and CD40L fluorescein isothiothiocyanate (Ancell Croup). Isotype-matched fluorescein isothiocyanate- and phycoerythrin-conjugated mouse IgG (Pharmigen) were used as negative controls. After incubation with or without PMA (32 nM)+A23187 (1 ug/mL) for 4 hours, each sample was treated with 2 mL of lysing solution of fluorescence-activated Cell sorter (FAScan) (Becton Dickinson), washed twice with cold PBS, resuspended in PBS with 1% paraformaldehyde, and analyzed using an FAScan. A total of 10000 cells were acquired and analyzed using CellQuest software (Becton Dickinson). The boundaries between stained and unstained populations were set using the isotype control settings, such that <1% of the events in the control tube were scored as positive. All experiments were carried out in triplicate. We also used thrombin (0.5/ml) as a stimulant in the CD40L/CD61 study. As shown in Table 1 and Figure 2, after PMA+A23187 stimulation, there were no significant increases in CD40L expression on the patient's $CD4^+$-T cell (resting vs. PMA+A23187 = 5.70% vs. 6.80%) compared with that of the age match control group (resting vs. PMA+A23187 = 12.40% vs. 58.30%). In the CD40L/CD61 study, after thrombin stimulation, the patient had no obvious expression of CD40L on platelets [resting vs. thrombin = 22.4 vs. 28.7 MFI (Mean fluorescence intensity)] however, the age matched control group had significant increases of CD40L expression on platelets (resting vs. thrombin = 42.7 vs. 120.3 MFI).

HIM with CD40L deficiency was proven.

An important consequence of many immunodeficiencies is the susceptibility to recurrent opportunistic infections. *Pneumocystis carinii* infections have been noted in HIM patients as well as in CD40L-deficient mice. It has been shown that interaction between T and B cells via the CD40-CD40L is essential for the resolution of PCP in mice.[16] Children with HIM are at an increased risk for PCP. Patients with PCP can present with fever, cough, respiratory distress, increasing hypoxia and eosinophilia. The CXR reveals bilateral diffuse alveolar pattern. The earliest densities are perihilar and progression proceeds peripherally. The definite diagnosis requires the demonstration of *P. carinii* in the lung. Methods for obtaining appropriate specimens include bronchoalveolar lavage, tracheal aspiration, transbronchial lung biopsy, bronchial brushing, percutaneous transthroracic needle aspiration, and open lung biopsy.[17] Our patient had fever, cough, rhinorrhea and tachypnea initially. Leucocytosis with eosinophilia (WBC: 30620/mm^3; eosinophils: 48%) was noted and CXR showed interstitial alveolar pattern with perihilar haziness over bilateral lung fields. Although his family refused further invasive evaluation for *P. carinii*, TMP-SMZ was used under the impression of PCP. CXR was followed up and revealed improvement afterward. Unfortunately, he suffered from peritonitis with ileum perforation after the improvement of lung condition. He received surgical treatment and the pathological findings of the surgical specimen showed lymphocyte infiltration. Peritonitis as the presenting manifestation of HIM is extremely rare. Benkerrou et al. had observed the massive intestinal lymphoproliferation which may result in peritonitis from the HIM patients.[4] In this case, we also found the same pathologic finding. Therefore, peritonitis, which may result from massive intestinal lymphoproliferation, although rare, can be a manifestation of HIM.

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免疫不全併高值IgM症候群合併間質性肺炎和腹膜炎

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免疫不全併高值IgM症候群是一種少見的疾病，爲CD40結合體基因突變所致.其血清IgM值常偏高或正常，而IgG、IgA及IgE則偏低。本文報告一例疑似肺囊蟲引起間質性肺炎的病童，經流速細胞計數法證實爲免疫不全併高值IgM症候群。經過靜脈注射免疫球蛋白及磺胺類抗生素治療後，肺炎情況明顯改善。隨後卻併發腹膜炎並且接受手術治療，病理顯示腸淋巴增生現象。回顧相關文獻得知免疫不全併高值IgM症候群合併腹膜炎極其少見，但由本病例可知腸淋巴增生所導致的腹膜炎仍是免疫不全併高值IgM症候群可能的併發症。(長庚醫誌 2003;26:53-9)

關鍵字：低丙種球蛋白血症，免疫不全併高值IgM症候群，CD40結合體，間質性肺炎，腹膜炎。

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