Serial Measurement of Soluble Interleukin 2 Receptor Levels: An Early Indicator of Treatment Response for Lyme Disease

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Abstract. **Detection of antibodies produced in response to infection with *Borrelia burgdorferi* provides a valuable aid for diagnosing Lyme disease. However, anti-Borrelial antibody titers are of little value in determining treatment success or providing evidence of persistent infection as levels of specific antibodies can remain elevated even after successful treatment. Pretreatment and posttreatment measurement of soluble interleukin 2 receptor (sIL-2R) levels was evaluated for use in predicting treatment response in Lyme disease. Results indicate that serial measurement of serum sIL-2R levels can provide an early indicator of response to treatment and outcome.** (*J Rheumatol 1993;***20:996–8**)

Key Indexing Terms:
sIL-2R SERIAL MEASUREMENT EARLY RESPONSE INDICATOR LYME DISEASE

Infection with *Borrelia burgdorferi*, the causative agent of Lyme disease (LD), can result in multisystem disorders with protean clinical manifestations[1,2]. Treatment with a single course of appropriate antibiotics effects a cure in most patients. However, some patients develop persistent or recurrent disease symptoms[3]. It is unresolved at present whether such patients have failed antibiotic therapy or have developed an autoimmune response independent of the presence of viable spirochetes[4]. Furthermore, as levels of anti-Borrelia antibodies tend to remain elevated for prolonged periods, serial anti-Borrelial antibody measurements are not useful to identify or follow patients (in the short term) who may develop chronic LD[5].

Proliferation assays, measuring T cell responses to *B. burgdorferi*, have been investigated as a possible means of detecting early LD and to determine if patients with LD have active infections[6,7]. Performing T cell proliferation assays requires considerable laboratory support, use of radioisotopes and long (6–7 day) incubation times. As a result such assays are not amenable to routine use in many clinical laboratories.

We examined the use of soluble interleukin 2 receptor (sIL-2R) level measurements, which are known to be correlated with T cell activation, as a method to determine active infection and response to treatment for patients with LD[8,9].

Results obtained showed that infection with *B. burgdorferi* is associated with elevations in serum sIL-2R levels. Following treatment with antibiotics, serum sIL-2R levels were reduced relative to pretreatment levels, and observed reductions appear to correlate with a successful response to treatment.

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MATERIALS AND METHODS

Patient selection. Experimental sera for our study was obtained from pediatric patients (13 males, 9 females) with clinically diagnosed, serologically confirmed LD who met the Centers for Disease Control criteria for LD and were being treated and followed at our institute's Lyme disease clinic. Ages for the LD patients range from 1.5 to 19 years with a mean of 8.4 ± 4.1 SD. Of the 22 patients in the study 18 were classified as having chronic Lyme arthritis, 3 acute localized erythema chronicum migrans (ECM), and 1 acute disseminated (Bell's palsy with history of ECM). Enrollment in the experimental group required at least one sample be available both pretreatment and posttreatment. All specimens were stored as serum at −70°C until run in batch for sIL-2R levels. Lyme serologies (Western blot for IgG and IgM antibodies and enzyme linked immunosorbent assay, ELISA) were performed prior to freezing specimens at the time of collection as described elsewhere[10,11]. Control sera for determining normal pediatric sIL-2R levels were obtained from patients (15 males, 34 females) participating in an autodonor program before elective orthopedic surgery. Mean age for controls was 14.8 ± 2.7 SD with a range of 9–19 years.

Determination of response to treatment was made using the following criteria: acute localized — absence of rash, fever or fatigue at treatment completion; acute disseminated — unquestionable and significant improvement on examination at completion of therapy and absence of findings at 12 weeks; Lyme arthritis — unquestionable and significant reduction of swelling of the affected joint(s) at completion of therapy with disappearance of any swelling at 12 weeks and no new joint recruitment at completion of therapy; nonresponder — response criteria not met.

sIL-2R assay. Samples were assayed for sIL-2R using T Cell Sciences (Cambridge, MA) Cellfree Interleukin 2 Receptor Test Kit. The assay was performed according to manufacturer's instructions. Briefly, 50 μl of sample

was added to polystyrene wells that had been precoated with a murine monoclonal anti-IL-2R antibody. Peroxidase conjugated mouse antihuman IL-2R monoclonal antibody with a different epitope specificity was added immediately after the sample and incubated for 3 h. Wells were washed with phosphate buffered saline and color was developed using O-phenylenediamine substrate. The reaction was stopped using 2N H_2SO_4 and the absorbance measured at 490 nm using a Titertek Multiscan (Flow Laboratories, McLean, VA). Unknown values were determined from the standard curve.

RESULTS

Data obtained from the measurement of sIL-2R levels in serial serum samples from patients with LD are depicted in Figure 1. Of the 22 patients with LD tested 20 were found to have elevated levels of sIL-2R in pretreatment sera relative to controls. Posttreatment or followup sera levels of sIL-2R were reduced relative to pretreatment levels for those 20 patients. The mean level of sIL-2R in pretreatment sera was 1457 U/ml ± 120 SE, posttreatment sera mean value was 1005 U/ml ± 131 SE and in followup sera 822 U/ml ± 81 SE. The time interval between pre and posttreatment samples ranged from 4–12 weeks ($\bar{x}$ = 6.4 ± 2.4 SD). The mean time in months for final serology (followup) testing was 5.8 ± 4.2 SD. The time interval for clinical followup ranged from 10 to 38 months ($\bar{x}$ = 24.7 ± 12.6 SD). Values obtained for 9 patients with separate followup sera (a 3rd specimen) were 1483 U/ml ± 185 SE pretreatment, 1157 U/ml ± 276 SE posttreatment and 709 U/ml ± 103 SE at followup. Statistical analysis (paired t test) showed a significant difference $p < 0.01$ between pre and posttreatment levels of sIL-2R and for pre and followup levels $p < 0.0001$ for the whole (n=22) group.

Data presented in Table 1 show sIL-2R levels determined from sera of 3 patients who initially failed to respond to treatment with antibiotics. Results of measuring sIL-2R levels on these 3 patients showed an increase (for 2 patients) following a first course of antibiotics. Following a 2nd course of antibiotics all 3 patients responded to treatment and serum sIL-2R levels were reduced by over 50% relative to pretreatment levels.

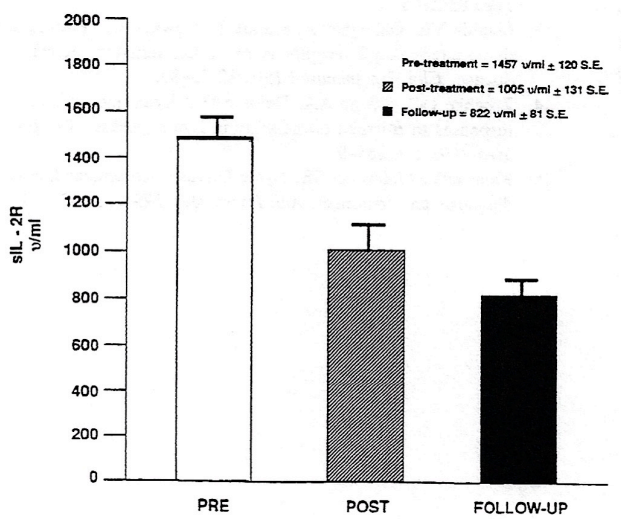

Fig. 1. The mean pretreatment (pre), posttreatment (post), and followup, serum levels of sIL-2R. Serum levels were significantly lower in post ($p < 0.01$) and followup serum ($p < 0.0001$) than those measured in pretreatment serum. Statistical analysis was performed using a paired t test.

Table 1. *sIL-2R levels in patients receiving 2 courses of antibiotics*

Patient	sIL-2R U/ml			
1	976	669	1069*	567†
2	2253	1180*	663†	
3	1105	3031*	672†	

* 2nd course of antibiotics administered following date at which sample was obtained. Clinical outcome nonresponder.

† Samples obtained after completion of 2nd course of antibiotics. Clinical outcome responder.

Results of measuring antibodies to *B. burgdorferi* by ELISA are shown in Table 2. Of the patients tested, 50% showed a decrease in antibody levels after successful treatment while 32% showed no change and 18% had increased serum levels of antibodies to *B. burgdorferi* following successful treatment. Erythrocyte sedimentation rates (ESR) were available for 17 of the 22 patients before treatment; 47% had ESR of 0–20, 12% 21–40 and 41% ≥ 41 mm/h. Because over half of the patients with untreated Lyme borreliosis had ESR within normal range, posttreatment ESR were not obtained.

Control sera to determine sIL-2R levels in a healthy pediatric population was obtained from autodonor blood samples of pediatric orthopedic patients prior to elective surgery. Mean values of sIL-2R in control sera (n=49) was 512 U/ml ± 19 SE.

DISCUSSION

Our results show that active infection with *B. burgdorferi* is usually associated with elevated serum levels of sIL-2R and that a decrease in sIL-2R levels following treatment with antibiotics appears to correlate with response to treatment. Earlier reports demonstrated a similar correlation for serum sIL-2R levels with disease activity in patients with rheumatic disorders and inflammatory bowel disease[12,13]. Clinical followup evaluations indicated that all 22 patients responded successfully to antibiotic therapy (although 3 required a 2nd course of treatment). Additionally, all 20 patients with elevated pretreatment levels of sIL-2R had decreased levels following successful treatment. Two patients of the 22 followed did not have significantly elevated levels of sIL-2R in serum obtained prior to treatment at the time of diagnosis. Both of those patients were classified as having Lyme arthritis with joint swelling at the time of diagnosis. It is unclear why these patients did not have elevated levels of sIL-2R in their pretreatment sera. Neither patient showed a significant change in serum sIL-2R levels after treatment and both were classified as responders to treatment.

Table 2. *Serum antibody levels pre and posttreatment*

Number Tested	$\bar{x}$ Antibody Titer		Number of Sera		
	Pre	Post	Increased	Decreased	Same
22	1364±288 SE	1204 ± 523 SE*	4	11	7

* There was no significant difference detected between pre and posttreatment levels of antibodies.

Assays measuring the proliferative response of T lymphocytes to *B. burgdorferi* are sometimes used to aid in diagnosing LD. These assays may be particularly useful when testing patients with early LD, who may not have produced sufficient levels of specific antibodies for detection by standard serologic methodologies[7]. Proliferation assays are also used to indicate infection in patients who, due to spirochete sequestration or other mechanisms, may not produce antibodies to *B. burgdorferi* in spite of longterm infection[7]. More recently T cell proliferation assays have also been found to be useful at distinguishing active from past infections[6]. The specificity of T cell proliferation assays appears, at least in some laboratories, to be low[14]. The potential for low specificity coupled with the degree of expertise and labor required to conduct these tests limits their utility for routine use[15].

We hypothesized that measurement of sIL-2R levels would provide a simple cost effective means of monitoring T cell activation in patients with LD. Because sIL-2R levels become elevated in response to numerous infectious agents and inflammatory conditions measurement on a single specimen is of little diagnostic value. However, by performing measurements on serial samples, coupled with clinical evaluation and measurement of antibodies to *B. burgdorferi*, sIL-2R levels may provide a useful method to distinguish active from past infections, and perhaps more importantly, provide an early indicator of patient response to treatment. We are currently conducting a longterm prospective study to further assess the utility of measuring sIL-2R levels in patients with LD.

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