

Immunoglobulin subclass levels in patients with non-Hodgkin lymphoma

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Allergy/atopy has been suggested to protect against non-Hodgkin lymphoma (NHL) and specific IgE levels are decreased in patients with NHL. We speculated that all immunoglobulin subclass levels might be downregulated in NHL and examined levels of IgM, IgD, IgA, IgE, IgG and IgG₄ in 200 NHL patients and 200 age- and sex-matched controls. Patients with B-cell NHL of many types had consistently lower median immunoglobulin subclass levels than controls. In every subclass except IgD, about 10–15% of B-cell NHL patients had absolute levels below the 2.5 percentile of controls. Subclass levels correlated with each other and many patients had more than one significantly low level. Levels were lowest for IgG₄ and IgE. Patients with chronic lymphocytic leukemia/small lymphocytic lymphoma had especially low total IgE levels. In other B-cell NHL types, total IgE levels were decreased to a similar extent as other immunoglobulin subclasses. In conclusion, low IgE levels are only part of a more generalized loss of immunoglobulins of all subtypes in a wide variety of B-cell NHL types. Low immunoglobulin levels appear to be a consequence of B-cell NHL presence, and we speculate about molecular mechanisms that could reduce all immunoglobulin subclasses in B-cell NHL.

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Non-Hodgkin lymphoma (NHL) is a malignancy of the immune system but its relationship to immunity is complex. A prominent observation is that NHL incidence has been increasing steadily over the past several decades in the developed countries,¹ starting long before the abrupt increases of high grade lymphoma seen in association with the AIDS epidemic.² For the non-AIDS-related NHL types, this trend continued in the United States at least through 1998.² In Scandinavia, similar increases were observed into the early 1990s, but rates have stabilized in the past decade.³ Some investigators proposed to explain this increase by a 'hygiene' hypothesis, which postulated that the impact of delaying a common infection might be responsible for increasing lymphoma risk,^{4,5} but other explanations are plausible. It may be that, when naturally occurring mutations accumulate in undifferentiated, replicate-competent cells, lymphoma becomes more common.⁶ A corollary to the hygiene hypothesis is that allergic/atopic conditions are becoming more common in the population.^{7–10} Chronic immunologic stimulation by allergens may serve to differentiate the immune system. Many studies have attempted to correlate allergy/atopy history with risk of lymphoma, with conflicting results.^{11–17} However, allergy/atopy represents a range of diverse conditions, ranging from hay fever and asthma to drug reactions. Furthermore, these conditions vary greatly in severity and hence in their recognition and reporting. These variations may lead to different immune impacts. Nevertheless, on balance, the evidence seems to favor lower lymphoma risk in persons with a history of self-reported allergy/atopy.

Allergy/atopy reactions are characterized by high levels of specific IgE responses to particular allergens, a readily measured immune marker. Some investigators have examined specific IgE

levels as representing a more standardized approach to classifying persons with allergy/atopy than self-reported history.^{17–21} Again, although the findings have not been entirely consistent, IgE levels appear to be generally higher in controls than NHL patients, consistent with the hypothesis that allergy/atopy is associated with a reduced risk of lymphoma.^{17–21} Yet arguing strongly against a causal link, risks of lymphoma were not associated with either specific or total IgE in a large Swedish cohort study.²² Furthermore, we reported that specific IgE reactivity was detected with similar frequency in samples obtained from controls and from patients ≥ 5 years before lymphoma onset but with lower frequency at or near the time of NHL diagnosis,¹⁷ implying that the reduction in specific IgE was related to disease presence rather than etiology.

Two recent reports reported that IgM and IgA were also reduced in persons with NHL.^{20,21} IgG levels were not reduced in one study²⁰ but low in the other.²¹ If immunoglobulin levels of all types are suppressed in persons with NHL, lower IgE levels in cases than controls might reflect a general abnormality in immunoglobulin production in persons with NHL, rather than a specific anomaly related to IgE and allergy/atopy. To examine this hypothesis, we compared IgM, IgD, IgA, IgE, IgG and IgG₄ levels in NHL patients to age- and sex-matched controls.

Material and methods

Samples were obtained from subjects in the Scandinavian Lymphoma Etiology Study (SCALE), a population-based case-control investigation conducted in Sweden and Denmark during 1999 through 2002.^{6,17} Cases were patients with incident NHL [including chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)] in adults 18–74 years old. Overall, 81% of cases and 71% of eligible controls participated. Pathology was reviewed by highly qualified hematopathologists in each country. Many cases had already started on therapy by the time of interview and bleed (median: 2.8 months after biopsy). By subtype, stage (Ann Arbor classification), age, sex, and other measures, the cases were similar in Sweden and Denmark.⁶ Controls were persons drawn in the same study population and matched on sex and age (within 10 years). Serum samples from cases and controls were stored at -80°C until testing. Details have been previously described.^{6,17} Because allergy symptoms were self-reported in the questionnaire data, we used Phadiatop (Pharmacia Diagnostics, Uppsala, Sweden) test results, available from our previously reported publication on atopy,¹⁷ to identify persons with atopy in the current study. The Phadiatop test is an enzyme-linked immu-

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TABLE I – CHARACTERISTICS OF SUBJECTS BY DISEASE: MEDIAN AGE AT DIAGNOSIS, SEX, INCREASED SPECIFIC IGE AND BLOOD DRAW RELATIVE TO TREATMENT (RX) ONSET

	No.	Age (years) (median)	Males	Increased sample specific IgE ¹	Sampled after Rx onset
NHL type					
All B-cell NHL	182	59	57.1%	14.8%	51.1%
Diffuse large B-cell	69	57	60.9%	23.2%	65.2%
Follicular	40	58%	37.5%	12.5%	42.5%
CLL/SLL ²	26	67	65.4%	0.0%	11.5%
Mantle cell	14	61	71.4%	28.6%	71.4%
Marginal	13	62	61.5%	7.7%	38.5%
B-cell not specified	12	65	75.0%	8.3%	66.7%
Lymphoblastic	8	61	37.5%	0.0%	62.5%
T-cell NHL	18	59	61.1%	16.7%	38.9%
Controls	200	59	57.5%	15.0%	Not applicable

¹Specific IgE levels ≥ 35 kU/L in the Phaditop test (see text). ²Chronic lymphocytic leukemia/small lymphocyte lymphoma.

noassay which detects circulating specific IgE to any of 10 common inhalant antigens in a single assay and has been fully described elsewhere.¹⁷ According to the manufacture's criteria, reactivity ≥ 35 kU/L indicated atopy. The study was approved by institution review boards in both Sweden and Denmark.

The original study included 3,055 NHL cases and 3,187 controls in both Sweden and Denmark. For practical reasons, the current study examined immunoglobulin subsets in serum from 200 cases and 200 individually age-, sex-, and country-matched controls, half from each country. To determine if the effect might be related to therapy, by design half of the NHL patients in each country had samples obtained pretreatment and half after treatment had started (median time from treatment onset: 2.6 months). Within strata of country and pre-/post-treatment case-control pairs were chosen randomly to have a typical representation of the lymphoma types. Data from Sweden and Denmark were combined because the measured effects on immunoglobulin levels were similar.

Immunoglobulin levels were assayed at the Statens Serum Institut in Copenhagen. IgG, IgA, IgM and IgE concentrations were measured using near infrared particle immunoassays on the IMMAGE[®] Immunochemistry System platform (Beckman Coulter, Inc., Fullerton, CA) using Kits # 474620 (IgE), # 446400 (IgG), #446460 (IgA) and # 447610 (IgM) provided by Beckmann Coulter. IgG₄ was likewise measured on the IMMAGE[®] platform, but using the kits #LK009.IM from The Binding Site Ltd. (Birmingham, UK). All measurements were performed according to the manufacturers' instructions. IgE assay results were based on reference sera from the WHO Second International Reference Preparation for IgE (IRP 75/502). IgG, IgG₄, IgA and IgM measurements were based on reference sera from the International Federation of Clinical Chemistry (IFCC) International Reference Preparation for Plasma Proteins lot CRM 470. IgD was measured by an in-house ELISA using rabbit anti-human IgD A0093 (DAKO A/S, Denmark) for catching and rabbit-anti-human IgD-HRP P0165 (DAKO A/S) for detection. The calibrators for IgD U/mL used the British Research Standard 67/37 as the referent sample. For each immunoglobulin type, the distribution of the log-transformed level was unimodal in both cases and controls. For descriptive presentation, levels of each subclass were categorized by the quartile distribution found in controls. We then calculated odds ratios (ORs) using the top quartile level in controls as the referent category. For correlations between subclass levels, we used Spearman (rank order) correlations and p values. We also used logistic regression to assess trends in these levels for each subclass by disease type.

To take into account the correlation between the immunoglobulin levels, we chose multivariate linear regression analysis methods to test for statistical significance. After normalizing the crude immunoglobulin levels by log transformation, we calculated the expected log transformed levels in the cases, from linear regression of log (IgX) on age, sex and country in the controls. Subtract-

ing the expected from the observed yielded a multivariate outcome variable of immunoglobulin level deviations in the cases. We assumed the multivariate outcome variables for cases to conform to requirements for multivariate normal analysis.²³ All statistical analyses were made using SAS version 9 (SAS Institute Inc., Cary, NC). The multivariate analyses were made using SAS Proc GLM with Wilk's lambda as the test statistic. Finally, we assessed whether IgE levels differed in cases and controls with atopy, using linear regression of log IgE on age, sex, country, case-control status, atopy and the interaction between atopy and case-control status. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

The median time from therapy onset to sample collection in patients who had started treatment was 82 days for B-cell cases and 46 days for T-cell cases. In patients with B-cell NHL, post-treatment levels were slightly but not statistically significantly lower than in pretreatment levels ($p = 0.25$) and did not vary significantly by subtype ($p = 0.17$). We therefore present and analyze pre- and post-treatment levels combined.

Results

The cases included 182 B-cell and 18 T-cell NHLs (Table I). Among B-cell NHL, diffuse large B-cell (38%), follicular (22%) and CLL/SLL (14%) types were most frequent. Because of small numbers, the T-cell lymphomas were not subclassified but included 5 anaplastic, 4 peripheral, 3 angioblastic or angioimmunoblastic, 2 mycosis fungoides, 2 intestinal and 2 unspecified types. The median age at NHL diagnosis was 59 years and 58% were males. As assessed by Phadiatop testing,¹⁷ atopy was found in 15% of cases and 15% of controls. Within the NHL group, atopy was most common in patients with mantle cell (29%) or diffuse large B-cell (23%) NHL, whereas no cases of CLL/SLL or lymphoblastic NHL had atopy. Among both cases and controls with atopy, the median levels of total IgE in persons were 7-fold higher than in those not reporting such conditions.

Median levels of immunoglobulin subclasses in cases and controls are shown in Table II. Patients with B-cell NHLs had consistently lower median levels than controls for all immunoglobulin types ($p < 0.0001$), the proportions varying from 88% for IgD to 46% for IgG₄ (Table II). In contrast, T-cell NHLs had subclass levels that were generally similar to those in controls ($p = 0.85$), although IgE levels were also relatively the lowest (median: 61% of controls). Among B-cell NHLs, the greatest difference between cases and controls was seen in IgG₄ and total IgE. IgG₄ levels were consistently low in all B-cell NHLs, whereas IgE levels in CLL/SLL cases were significantly lower than in other B-cell NHL subtypes ($p = 0.001$). After excluding CLL/SLL, total IgE levels did not vary significantly among the remaining B-cell NHL subtypes ($p = 0.91$) and were generally decreased to a similar extent as other immunoglobulin subclasses.

TABLE II – CRUDE MEDIAN IMMUNOGLOBULIN LEVELS, BY NHL TYPE, AND PERCENTAGE (%) OF MEDIAN LEVEL TO MEDIAN LEVELS IN CONTROLS

	IgM (g/L)		IgD (U/mL)		IgA (g/L)		IgE (IU/mL)		IgG (g/L)		IgG ₄ (g/L)	
All B-cell NHL	0.72	79%	18.6	88%	1.66	71%	9.7	52%	8.8	79%	0.16	46%
Diffuse large B-cell	0.77	85%	17.4	82%	1.94	83%	15.2	82%	8.8	79%	0.16	46%
Follicular	0.72	79%	14.4	68%	1.34	57%	10.0	54%	8.0	71%	0.14	40%
CLL/SLL ¹	0.49	54%	26.7	126%	1.55	66%	5.0	27%	9.7	87%	0.13	37%
Mantle cell	0.60	66%	27.0	127%	2.33	100%	21.0	114%	8.9	79%	0.20	57%
Marginal	0.86	95%	25.3	119%	1.63	70%	13.7	74%	10.9	97%	0.16	46%
B-cell not specified	0.97	106%	21.7	102%	1.75	75%	13.0	46%	8.5	76%	0.17	49%
Lymphoblastic	0.70	78%	8.5	40%	1.11	47%	5.4	29%	6.3	56%	0.18	51%
T-cell NHL	0.78	86%	25.0	118%	1.93	82%	11.2	61%	11.9	106%	0.35	100%
Controls	0.91	100%	21.2	100%	2.34	100%	18.5	100%	11.2	100%	0.35	100%

¹Chronic lymphocytic leukemia/small lymphocyte lymphoma.

For levels in the 200 normal subjects, the 95% confidence intervals (assessed as the interval between the 2.5% percentile and the 97.5% percentile) were IgM: 0.31–3.26 g/L; IgD: 0.22–264.3 U/mL; IgA: 0.65–6.99 g/L; IgE: 5–738 IU/mL (5 being the lower limit of detection); IgG: 5.84–17.9 g/L; and IgG₄: 0.05–0.45 g/L. The proportion of B-cell NHL cases with levels below the lower limit (2.5%) observed in controls were: IgM: 11%; IgD: 2%; IgA: 14%; IgG: 16%; and IgG₄: 16%. In control subjects, 20 (10%) had IgE levels below the threshold of detection, precluding an analysis of values below 2.5% of levels in controls, but, in comparison with controls, 66 (37%) of B-cell NHL cases had levels of ≤ 5 IU/mL ($p < 0.0001$). Among B-cell NHL cases, 68% of CLL/SLL cases and 50% of lymphoblastic lymphomas had levels below the threshold of detection, compared with 31% of other NHL types ($p = 0.0004$ and $p = 0.27$, respectively).

Subtype levels tended to correlate with each other, except for IgD, with correlations being stronger in patients with B-cell NHL than in controls (Fig. 1). Too few T-cell NHL patients were tested to yield stable conclusions. Patients with B-cell NHL were more likely to have severe immunoglobulin reductions (below 2.5% of levels in controls) in at least one subclass than controls and often had more than one significantly low level of immunoglobulin subclass. Specifically, the distribution of having 0, 1, 2 or at least 3 subclass levels below the lower 95% confidence limit for any subclass (excluding IgE for the reasons described above) in controls was 176 (88.0%), 23 (11.5%), 1 (0.5%) and 0 subjects, whereas in B-cell NHL cases, the corresponding distribution was 117 (64.3%), 36 (19.8%), 21 (10.4%) and 9 (5.0%), respectively (trend difference: $p < 0.0001$).

To examine the association between degree of immunoglobulin loss and likelihood of having NHL, we stratified the samples according to the quartile distribution of immunoglobulin subclasses in controls and examined ORs of being a NHL case (reference stratum: the highest quartile). In all subtypes of immunoglobulin except IgD, NHL cases were significantly over-represented in the lowest quartiles (Fig. 2). Although patients with more advanced disease, as measured by stage, had lower levels in every subclass, the trend tests by disease stages I to IV were statistically significant in only a few: IgM ($p_{\text{trend}} = 0.09$); IgD ($p_{\text{trend}} = 0.86$); IgA ($p_{\text{trend}} = 0.04$); IgE ($p_{\text{trend}} = 0.27$); IgG ($p_{\text{trend}} = 0.13$); or IgG₄ ($p_{\text{trend}} = 0.05$).

Discussion

Previous studies have reported lower specific and total IgE levels in NHL patients than controls and suggested that this finding supports lower NHL risk in persons with allergy/atopy.^{18,21} We confirm here that total IgE is lower in cases than controls, particularly in patients with CLL/SLL. However, we found that all other immunoglobulin subclass levels were also low in B-cell NHL cases. Relative to other immunoglobulin subclasses, total IgE did not appear to be particularly low, except in patients with CLL/SLL and perhaps lymphoblastic tumors. Thus, the lower

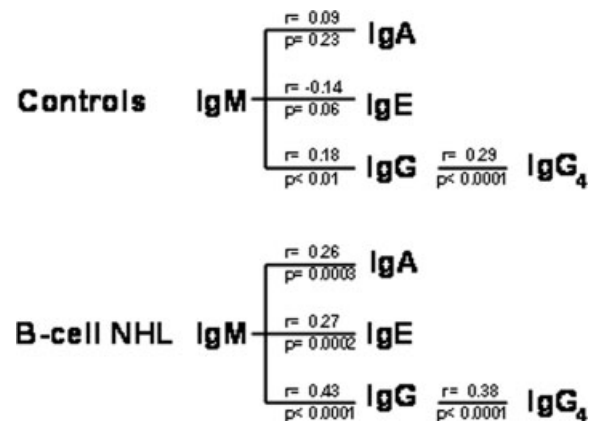


FIGURE 1 – Spearman (rank order) correlations between immunoglobulin subclass levels in healthy controls and patients with B-cell NHL. IgM relationships to IgD are not shown but were not statistically significant. Evaluation of the relationship between IgM and IgE was limited by the test sensitivity at low levels (see text).

level of IgE in cases may be only part of a mild generalized immunoglobulin suppression in patients with B-cell NHL. In contrast, immunoglobulin levels in T-cell NHL patients were not generally lower than expected, using a methodology that takes into account the multiple correlated outcomes. No significant associations were observed for IgD, but the IgD assay we used was designed to detect hyper-IgD syndromes, and the results may have been unreliable in the low levels seen in these subjects.

Our findings therefore confirm other recent reports of significantly lower levels of IgM and IgA.^{20,21} Additionally, we found lower IgG levels in NHL patients, in agreement with one report²¹ but not the other.²⁰ We extend these findings by observing reductions in additional immunoglobulin subclasses and by analyzing subclass results in a variety of lymphomas to show that the decreased IgM, IgA, IgG and IgG₄ levels are a consistent finding in all B- but not T-cell NHL subtypes. Patients with more severe disease had lower levels of all immunoglobulin subclasses, consistent with a prior report.²⁰ In the current study, levels were consistently but modestly lower (not significantly) in B-cell NHL patients who had started therapy (average time on therapy 82 days). In contrast, in another study, levels were higher after therapy had been started,²⁰ but the time since therapy onset was not described in that study.

Although the median immunoglobulin subclass levels were relatively low compared with controls, the majority of B-cell NHL patients still had subclass levels that were within the 95% range of levels in healthy controls. Levels of immunoglobulin in the range of normal seem unlikely to greatly affect susceptibility to infection. However, excluding IgE, 36% of the B-cell NHL patients

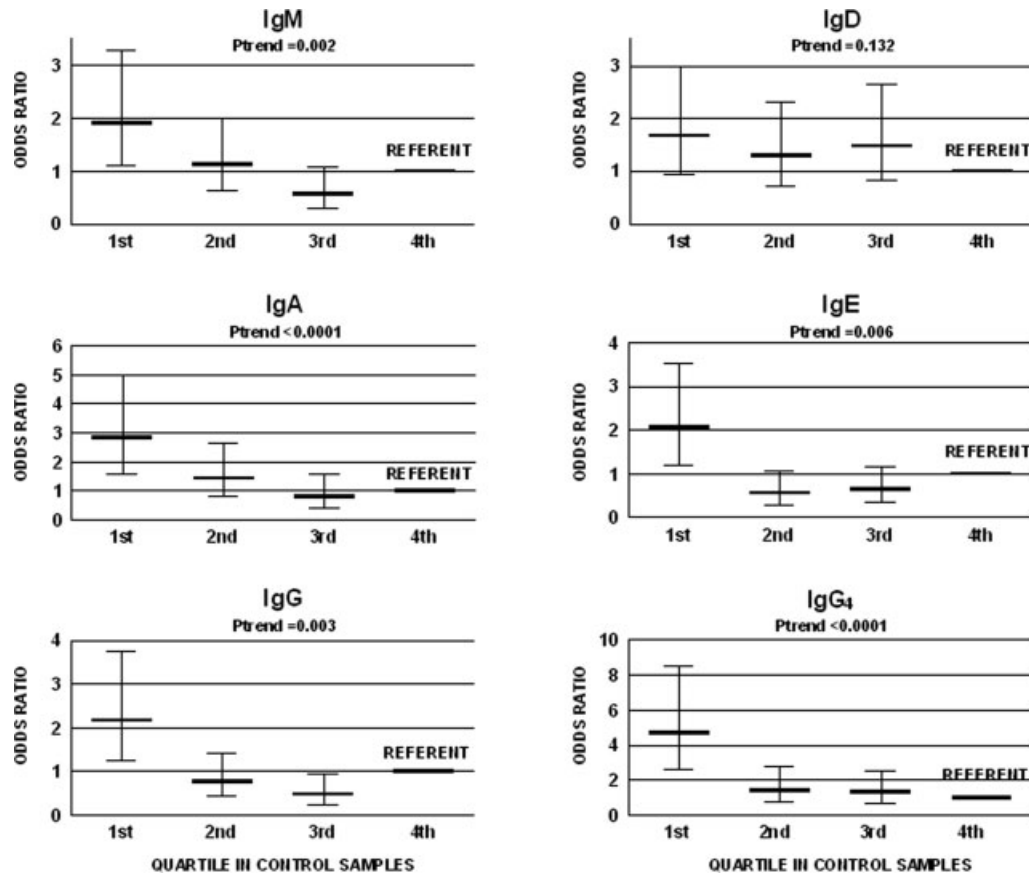


FIGURE 2 – Immunoglobulin subclass levels in B-cell NHL patients distributed by the quartile distribution of levels in healthy control subjects. Odds ratios and 95% confidence intervals are indicated. In all subtypes of immunoglobulins except IgD, NHL patients were significantly over-represented in the lowest quartiles of the distribution found in controls.

had at least one subclass level that was below the 2.5% lower limit seen in controls and 20% had two or more subclass levels that were significantly low, which might be clinically relevant. For IgE, levels were below the limit of detection in 10% of controls but 37% of cases. Patients with CLL/SLL or lymphoblastic lymphomas had particularly low total IgE levels and none of the patients with either B- or T-cell NHL type had high specific IgE levels. Thus, the inverse relationship between allergy/atopy and NHL,^{17–20} assessed at a time when lymphoma was present, is likely to be one aspect of a generalized immunoglobulin suppression.

Our findings raise the issue of whether low immunoglobulin levels is involved in lymphomagenesis, as proposed elsewhere,²⁰ or only a consequence of tumor presence. Because we lacked samples from before tumor onset, we cannot determine if decreased levels preceded the development of NHL. We favor decreased immunoglobulin levels being a consequence of disease presence, because (i) we find low levels in all subclasses of immunoglobulins, except IgD; (ii) we find a similar high frequency of low subclass levels in many B-cell NHL types; (iii) the inverse association with atopy was not seen until at or just before NHL onset in our earlier study that included prospective samples¹⁷; (iv) neither specific nor total IgE levels predicted lymphoma risk in a large cohort study²²; and (v) levels were lower in NHL patients with more advanced disease both for most immunoglobulin subclasses, including total IgE, as tested here, and for specific IgE testing (Phadiatop testing), as presented earlier.¹⁷ Although the association with stage was weak and only sometimes statistically significant in the different immunoglobulin subclasses tested in our study, stage is an imperfect marker of the extent of disease and its meaning will vary according to the subtype of NHL being considered. Others have also reported a simi-

lar association between more advanced stages of NHL and lower levels of immunoglobulins.²⁰

If the low immunoglobulin levels are a consequence of having active B-cell NHL, how might this happen? In Sweden and Denmark, patients with NHL generally present before they become wasted from their illness, making it unlikely that immunoglobulin levels were low because of catabolism. We propose that it is more likely that downregulation occurs because of impaired immunoglobulin production in all subclasses rather than subclass switching. Immunoglobulin is produced by B cells acting under T-cell influences. Antigen stimulation is critical to antibody production, but, as a mechanism to prevent auto-reactivity, the Th2 immune response also requires interactions between antigen presenting cells and T-lymphocytes by co-stimulating regulatory proteins on cell surfaces. One example is the attachment to CD28 receptors on T-cells by members of the B7 family.²⁴ B7-1 (CD80) and B7-2 (CD86) are both expressed on B cells and interact with T cells, resulting in up-regulation of T-cell surface molecules such as CD40L. Although the net result is normally to stimulate greater antibody responses as well as to promote immunoglobulin subclass switching,²⁵ these two surface proteins have opposing effects. Whereas B7-2 (CD86) enhances B cell activity, B7-1 (CD80) downregulates immunoglobulin production.²⁶

A possible mechanism of downregulation could be that the soluble CD80 (sCD80) release from B-cell lysis results in suppression of immunoglobulin production. Such a mechanism would predict effects in persons particularly with active or with more advanced stages of disease. It could also explain the modest reductions of immunoglobulin levels in patients undergoing treatment compared with pretreatment levels, although we acknowledge that the toxic effects of systemic therapy could also directly interfere with

immunoglobulin production. By NHL type, immunoglobulin levels were generally lowest in patients with CLL/SLL. In further support, patients with CLL/SLL have apparently functional sCD80 detected more frequently than controls, and higher levels of sCD80 were correlated with poor prognosis.²⁷ However, sCD80 interactions with T-cells may not be the only mechanism lowering immunoglobulin production. In CLL/SLL, soluble CD27 also

inhibits T-cell responses to antigen presenting cells through an effect on CD70.²⁸

In summary, all immunoglobulin subclass levels tended to be low in most B-cell NHL subtypes, indicating a general mechanism by which production may be impaired by the presence of B-cell NHL. The mechanisms by which this suppression might occur in patients with NHL deserve further exploration.

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