Immune function in multiple myeloma:
Impaired responsiveness to keyhole limpet hemocyanin

JULES HARRIS, M.D., F.R.C.P.[C], F.A.C.P., RAYMOND ALEXANIAN, M.D., EVAN HERSH, M.D. and PHILIP MIGLIORE, M.D., *Houston, Texas, U.S.A.*

Summary: Twenty-three patients with multiple myeloma, four patients with treated localized plasmacytoma and 14 normal subjects were immunized with keyhole limpet hemocyanin (KLH). When compared to the normal subjects, the myeloma patients showed a prolonged induction time for IgM antibody formation, a more rapid switch from IgM to IgG production and a decline in the titre of total antibody produced. In vitro *lymphocyte responses to KLH following immunization were reduced in the myeloma group and tended to decline with time in a manner similar to the serum antibody concentration. Most of the myeloma patients tested developed delayed hypersensitivity skin reactions to KLH, but these reactions were smaller than those of the control subjects. The patients with myeloma had also reduced* in vitro *lymphocyte responses to streptolysin-O and vaccinia. Immune function of the plasmacytoma patients was similar to that of the control subjects.*

Both humoral and cellular immunity in response to a newly encountered antigen, KLH, is impaired in patients with multiple myeloma.

The high incidence of infectious complications in patients with multiple myeloma has prompted a number of studies of adaptive immunity in these patients.[1-4] A significant relationship has been reported between abnormal antibody response and susceptibility to infection.[2] Most patients with multiple myeloma have demonstrable established delayed hypersensitivity to at least one of the commonly encountered antigens, e.g. tuberculin, streptokinase-streptodornase, but may be incapable of developing delayed hypersensitivity to new antigens such as dinitrofluorobenzene.[4] Until recently it was thought that phytohemagglutinin induced normal stimulation of *in vitro* blastogenesis in lymphocytes from myeloma patients. Using synchronized cell culture systems, however, even this index of immunity has been found to be quantitatively decreased.[5] The precise mechanism of the immunologic unresponsiveness in myeloma remains to be defined. The investigation reported below approached this problem by immunizing a group of multiple myeloma patients with keyhole limpet hemocyanin (KLH). This antigen is particularly useful in that a single immunization will elicit in man antibody of IgM and IgG type, *in vivo* delayed hypersensitivity and *in vitro* lymphocyte blastogenesis.[6, 7] Study of the concurrent development of these various parameters of adaptive immunity might be expected to shed additional light on the immune deficiency state associated with multiple myeloma. The result of greatest interest which emerged from this study was that patients with myeloma, when immunized with KLH, have a prolonged induction time for the production of IgM antibody but a more rapid shift from IgM to IgG antibody formation when compared to normal subjects.

Materials and methods

Patients

Twenty-three patients with multiple myeloma were studied at the M. D. Anderson Hospital, Houston, Texas, between January and July, 1968 (Table I). The diagnosis of multiple myeloma in each case was established by examination of bone-marrow aspirates and study of serum and urine proteins. In the majority of instances, patients were studied immediately after the diagnosis had been made and prior to any chemotherapy. Patients who had received chemotherapy were studied at least one to two weeks after their last course of drugs. Some patients while on study received local radiation to painful bone lesions. No patient received radiation either to lymph node-bearing areas or to spleen during the investigation period. Normal subjects were chosen from the medical and technical staff of the M. D. Anderson Hospital; all were male and their ages ranged from 27 to 64 years (mean: 41 years). Four additional patients (three males, one female) with localized plasmacytoma successfully treat-

Presented in part at the Fiftieth Annual Session of the American College of Physicians, Chicago, Illinois, April 20-25, 1969.

From the Department of Developmental Therapeutics and the Department of Medicine, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, U.S.A.

Supported by contract 43-68-949, Collaborative Research Program, Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S.A.

Reprint requests to: Dr. Jules Harris, Department of Medicine, Faculty of Medicine, University of Ottawa, Ottawa General Hospital, Ottawa 2, Ontario.

ed by operation and/or radiation were also studied. They ranged in age from 48 to 81 years (mean: 61 years).

Immunologic methods

Live keyhole limpets were obtained from the Pacific Biomarine Company, Venice, California. Hemocyanin was extracted and purified according to the method of Campbell *et al.*[8] Patients and normal subjects were immunized with 5 mg. of KLH given subcutaneously in the deltoid area. Blood was drawn at weekly intervals thereafter, for both serum antibody determinations and study of *in vitro* lymphocyte blastogenesis. These evaluations were continued over a seven-week period.

Serum antibodies were measured by passive hemagglutination using the tanned red cell technique.[9] Sensitivity to 0.1 molar 2-mercaptoethanol (2-ME) was used to distinguish between IgM(19S) and IgG(7S) antibodies.[10] Antibody titres are reported as $\log_2$ of the highest positive serial two-fold dilution.

The development of delayed hypersensitivity to KLH was assessed by the intracutaneous injection of 100 μg. of antigen seven days after immunization. When a negative result was obtained, the test was repeated at 14 days. Skin tests were read at 20 minutes, 1 hour, 24 hours and 48 hours. Induration was measured in two diameters at right angles, and the mean of these measurements is reported.

The lymphocyte culture technique was that of Hersh and Harris.[11] A total of 10^6 lymphocytes was cultured in vertical, Pyrex glass, round-bottom, screw-top tubes measuring 13 x 100 mm. Each culture tube contained 2 ml. of Eagle's Spinner Modified Minimal Essential Media and 1 ml. of autochthonous serum. The Eagle's media was supplemented with 20 mM/litre glutamine and contained penicillin - streptomycin solution. The *in vitro* response to KLH over a range 0.001 mg. to 0.2 mg. was studied. Control cultures were unstimulated. Additional cultures in each set were stimulated with the following antigens: 0.1 ml. vaccinia solution (Vaccinia, Dryvax–Wyeth, 1:100 dilution of standard vaccination suspension) and 0.1 ml. SLO (Streptolysin-O, Difco Laboratories). Cultures were incubated at 37° C. in an atmosphere of 5% carbon dioxide in air for five days. Following the five-day culture period, 2 microcuries of ^{3}H-thymidine (specific activity 6.7 curies per millimole–New England Nuclear Corporation) were added to each tube and an additional three hours of incubation were allowed. The cells were then washed twice with saline. The acid-insoluble radioactivity was precipitated twice with 5% trichloroacetic acid and the specimens processed for liquid scintillation counting. Thymidine incorporation is reported as counts per minute per 10^6 lymphocytes in culture. The result for stimulated cultures was obtained by substituting thymidine incorporation in the appropriate unstimulated controls.

Results

Antibody production

Antibody responses following primary immunization are illustrated in Fig. 1. No antibody to KLH was present in the serum of either patients or controls prior to immunization. Whereas control subjects developed antibody to KLH by the seventh postimmunization day, the myeloma patients had in most instances no detectable antibody in their serum until day 14. At day 7 only eight out of 23 patients had detectable antibody of any type. Of these eight, six produced both IgM and IgG antibody. By day 14, 22 of the 23 patients were producing some antibody and 20 produced both IgM and IgG types of antibody. No IgG antibody was produced by any member of the control population earlier than at least 14 days after immunization. IgG is detectable at day 28. A peak median titre for total antibody of 4 (dilution 1-16) is reached in the myeloma group. This compares to a value of 6 (dilution 1-64) for the normal subjects. There is a decline in total antibody titre after day 14 in the myeloma group. The total antibody titre of the control group plateaus to at least 56 days after immunization. The humoral immune responses of the plasmacytoma group were in the normal subject range.

Delayed hypersensitivity

The results obtained following skin testing with KLH at seven days after immunization are given in Table II. All normal subjects had developed delayed hypersensitivity to KLH by day 7. The mye-

TABLE I
Clinical information regarding patients studied

Age and sex	
11 males	—median age 62 years
	—(range 56-81 years)
12 females	—median age 62 years
	—(range 39-78 years)
Myeloma protein type	
ϒ G	K —8 (35%)*
	L —6 (17%)
ϒ A	K —2 (10%)
	L —3 (13%)
BENCE JONES PROTEIN only —	
	K — 3 (13%)
	L — 1 (12%)

*Percentages in parentheses refer to the distribution of myeloma protein types in patients with multiple myeloma seen at the M.D. Anderson Hospital in the 10-year period prior to this study.

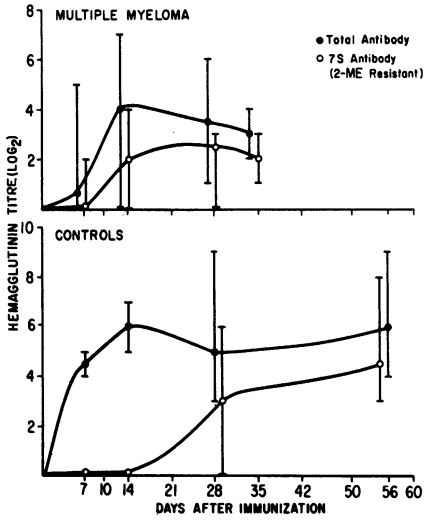

FIG. 1—A comparison of humoral antibody formation in normal subjects and myeloma patients after KLH immunization. The vertical bars indicate ranges. Lines are drawn through median values.

TABLE II
Delayed hypersensitivity after KLH immunization

	Normal subjects	*Myeloma patients*
Number tested	14	10
Number positive	14	8
Median induration (mm.)	10.3	6.5
Induration range (mm.)	5 — 30	0 — 15.5

loma patients gave positive reactions in eight of 10 patients tested. The two patients with negative tests at seven days were tested again at day 14 and were positive at that time. The median induration of reaction measured in the myeloma patients was less than that in the control group.

All 10 of the myeloma patients studied had established delayed hypersensitivity reactions, 5 mm. of induration or greater, at 48 hours to at least one of the following skin test antigens: Candida 1/50 (Dermatophytin "O", Hollister-Stier Laboratories), Trichophyton 1/50 (Dermatophytin, Hollister - Stier Laboratories), streptokinase-streptodornase 10 units (Varidase, Lederle Laboratories) and intermediate-strength purified protein derivative (P.P.D., 0.0002 mg., Parke-Davis and Co.)

In vitro *lymphocyte blastogenesis*

The *in vitro* blastogenic response to KLH stimulation by lymphocytes taken both from control subjects and myeloma patients was followed serially in the postimmunization period. The results are illustrated in Fig. 2. Both the control and the myeloma patients showed a slight but definite response *in vitro* to KLH at day 0 prior to immunization. The ability of the myeloma group to respond

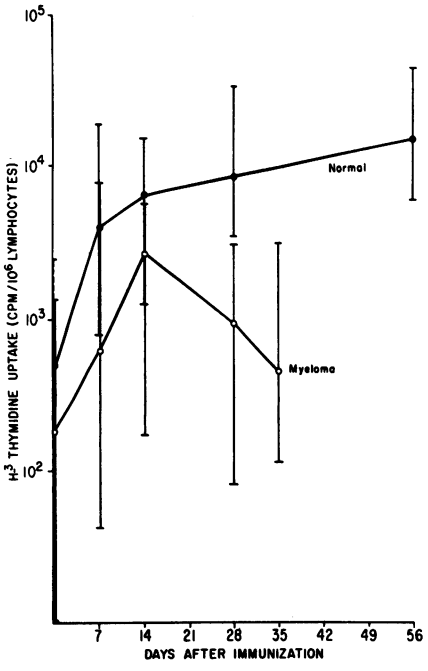

FIG. 2—A comparison of *in vitro* lymphocyte blastogenesis in normal subjects and myeloma patients after KLH immunization. The vertical bars indicate ranges. Lines are drawn through median values.

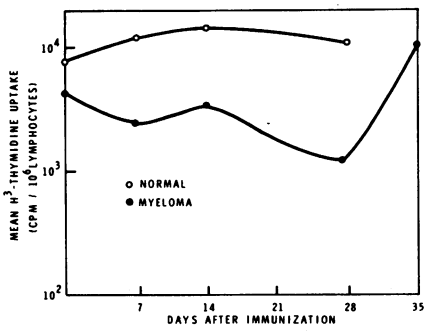

FIG. 3—A comparison of *in vitro* lymphocyte blastogenesis following stimulation with SLO in normal subjects and myeloma patients. Lines are drawn through median values.

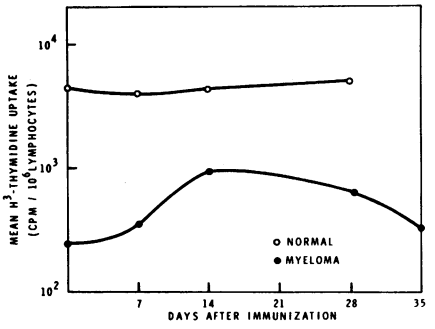

FIG. 4—A comparison of *in vitro* lymphocyte blastogenesis following stimulation with vaccinia in normal subjects and myeloma patients. Lines are drawn through median values.

in this way was impaired when compared with the controls—a median value of 180 counts per minute (cpm) as opposed to a median of 500 cpm. The responses increased in both groups in the two-week period following immunization. There was some overlap of ranges, but the responses remained quantitatively distinct. At 14 days the median response for the myeloma group was 2700 cpm compared to 6800 cpm for the controls. The control response tended to plateau thereafter—out to 56 days post immunization and beyond. Between 14 and 28 days after immunization, however, the myeloma response fell off sharply and continued to decline out to 35 days when the last serial sample for the myeloma group was obtained. By 35 days the KLH response had dropped to 600 cpm, almost back to the preimmunization level.

The patients with myeloma were found to have impaired *in vitro* lymphocyte responses to both SLO (Fig. 3) and vaccinia (Fig. 4) during the course of the study. The mean response to vaccinia for the myeloma patients was 5.4 x 10^2 cpm (compared to 5 x 10^3 in the normal subjects). The mean response to SLO in the myeloma group was 4.6 x 10^3 (compared to 1.2 x 10^4 in the normal subjects). The response to these mitogenic agents did not change appreciably in either group during the period following KLH immunization.

The *in vitro* lymphocyte responses to KLH following immunization in the group of four plasmacytoma patients are illustrated in Fig. 5. Their responses approximate those of the control subjects and show none of the decline noted in the myeloma group after day 14-21.

No relationship could be established in this study between the immune responses measured and the following variables: age, sex, previous treatment, immunoglobulin levels, height of myeloma peak and clinical condition.

Discussion

Static antibody determinations have shown that patients with myeloma have low levels of circulating antibody to a variety of common antigens.[12, 13] This is in part due to the impaired gamma globulin formation and increased gamma globulin catabolism known to occur in myeloma patients.[14] Depressed humoral immune responses to test antigens have also been found in myeloma.[1, 2] The primary immune response is more severely affected than the secondary response.[4] Cell-mediated immunity is also impaired.[4] The lymphocytes from patients with

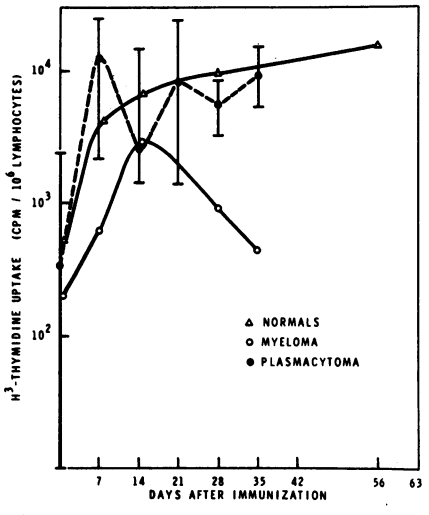

FIG. 5—A comparison of *in vitro* lymphocyte blastogenesis in normal subjects, patients with treated localized plasmacytoma and myeloma patients after KLH immunization. The vertical bars indicate ranges. Lines are drawn through median values.

multiple myeloma respond poorly *in vitro* to phytohemagglutinin under conditions of cell culture synchronization.[5] Our study demonstrates poor *in vitro* blastogenic responses by myeloma lymphocytes to streptolysin O and vaccinia. These represent immune responses to antigens to which patients have had previous immune exposure (patients were not immunized with these antigens during the study). Our study adds a new dimension to the investigation of immune deficiency associated with human malignancy. The normal humoral immune response in man is marked by antibody formation first of IgM type with a switchover in time to antibody which is predominantly IgG in nature.[15] This change has not been adequately investigated in human disease. We have characterized the immune response to KLH in patients with myeloma as one which, compared to that in normal subjects, is marked by a generally prolonged induction time for IgM antibody but a more rapid switchover to IgG antibody formation. The decline in total antibody titre in the weeks following immunization may be due to increased gamma globulin catabolism. It may also be explained by the abnormal function or decline in number of "memory cells" responsible for antibody production. *In vitro* lymphocyte blastogenesis has been correlated with antibody formation in man.[16] A portion of the lymphocytes responding *in vitro* may be "memory cells" concerned with humoral antibody production.[17] The serial study of *in vitro* lymphocyte blastogenesis in response to KLH shows a fall-off in blastogenesis due to either reduction or impaired function of "memory cells".

We have no definitive explanation for the accelerated immunologic maturation from IgM to IgG antibody production in myeloma. Three possibilities might be considered. (1) The same abnormal stimulus which is driving abnormal lymphoblasts to abnormal plasma cells may be acting on the normal lymphoblast and plasma cell populations of our patients. This assumes that IgM antibody is largely the product of lymphocytes and lymphoblasts and that IgG antibody is largely the product of plasma cells.[18] (2) The total immunologic potential in our patients may be reduced by their disease to the point where some as yet undefined homeostatic mechanism acts to speed up the transition from IgM to IgG antibody production in the interests of immunologic efficiency. (3) The immune apparatus of the myeloma patient may be exquisitely sensitive to "feed-back" inhibition of IgM antibody production by IgG antibody.[19]

Solitary plasmacytoma frequently progresses to disseminated myeloma despite vigorous treatment to the localized lesion.[20] Patients with treated plasmacytoma had a normal immune response to KLH. This suggests that the immune deficiency which characterizes multiple myeloma probably does not precede the overt development of the disease but is a consequence of it.

Current concepts in immunology recognize two functionally distinct, although morphologically indistinguishable populations of small lymphocytes.[21] This hypothesis provides a useful basis for discussion. One cellular component is dependent for its development on the thymus and affects the cell-mediated immune responses of delayed hypersensitivity and allograft rejection. These lymphocytes are termed "thymus-dependent". The second population of small lymphocytes responds to antigenic stimulation with the formation of humoral antibody. Such lymphocytes are labelled "immunoglobulin-producing". The "memory cells" of both groups probably respond *in vitro* to specific antigens by undergoing blastogenesis. "Memory cell" function with regard to antigens presumably encountered prior to the onset of disease is relatively intact in patients with multiple myeloma. They have normal secondary humoral immune responses and well-preserved established delayed hypersensitivity reactions. Some impaired function or decrease in the number of these "memory cells" is suggested by our finding of reduced *in vitro* responses to vaccinia and SLO. Our experiments further demonstrate that immunologically competent lymphocytes of both "thymus-dependent" and "immunoglobulin-producing" types, together with the "memory cells" which subsequently develop from these cells following exposure to new antigens, are abnormal in patients with clinically overt multiple myeloma. These patients have qualitatively and quantitatively impaired primary humoral immune responses, a quantitative decrease in the intensity of newly developed delayed hypersensitivity reactions and abnormal *in vitro* lymphocyte blastogenesis to specific antigens.

The small but definite *in vitro* lymphocyte response to KLH seen in the preimmunization samples obtained from both patients and control subjects may have been an example of a primary immune response *in vitro*. Alternatively, it could represent an instance of antigenic cross-reactivity.[22]

References

1. ZINNEMAN HH, HALL WH: *Ann Intern Med* 41: 1152, 1954
2. FAHEY JL, SCOGGINS R, UTZ JP, et al: *Amer J Med* 35: 698, 1963
3. LINTON AL, DUNNIGAN MG, THOMSON JA: *Brit Med J* 2: 86, 1963
4. CONE L, UHR JW: *J Clin Invest* 43: 2241, 1964
5. SALMON SE, FUDENBERG HH: *Blood* 33: 300, 1969
6. CURTIS JE, HERSH EM, HARRIS JE, et al: *Clin Exp Immun* 6: 473, 1970
7. SWANSON MA, SCHWARTZ RS: *New Eng J Med* 277: 163, 1967
8. CAMPBELL DH, GARVEY JS, CREMER NE, et al: *Methods in Immunology*, New York, W.A. Benjamin, 1964, p 69
9. STAVITSKY AB: *J Immun* 72: 360, 1954
10. MITCHELL MS, WADE ME, DECONTI RC, et al: *Ann Intern Med* 70: 535, 1969
11. HERSH EM, HARRIS JE: *J Immun* 100: 1184, 1968
12. MARKS J: *J Clin Path* 6: 62, 1953
13. LAWSON HA, STUART CA, PAULL AM, et al: *New Eng J Med* 252: 13, 1955
14. SOLOMON A, WALDMANN TA, FAHEY JL: *J Lab Clin Med* 62: 1, 1963
15. BANDILLA KK, MCDUFFIE FC: *Fed Proc* 27: 563, 1968 (abstract)
16. LEVENTHAL BG, SMITH CB, CARBONE PP, et al: Lymphocyte transformation in response to *M. pneumoniae*, in *Proceedings of the Third Annual Leucocyte Culture Conference, University of Iowa, Iowa City, November 1967*, edited by RIEKE WO, New York, Appleton-Century-Crofts, 1969, p 519
17. BROSTOFF J, GREAVES MF, ROITT IM: *Lancet* 1: 803, 1969
18. NOSSAL GJ, SZENBERG A, ADA GL, et al: *J Exp Med* 119: 485, 1964
19. UHR JW, MOLLER G: *Advances Immun* 8: 81, 1968
20. COHEN DM, SVIEN HJ, DAHLIN DC: *JAMA* 187: 914, 1964
21. PETERSON RD, COOPER MD, GOOD RA: *Amer J Med* 38: 579, 1965
22. HARRIS JE, HERSH EM, HUGGINS S: *Transplantation* 9: 440, 1970

Résumé

Réaction immunitaire anormale à l'hématocyanine de patelle keyhole chez des malades souffrant de myélome multiple

Nous avons immunisé au moyen d'hémocyanine extrait de patelle keyhole (HPK) 23 malades souffrant de myélome multiple, quatre malades traités présentant des

plasmocytomes localisés et 14 sujets normaux. Par comparaison avec les sujets normaux, les malades atteints de myélome présentaient un temps d'induction prolongé dans la formation des anticorps IgM, un transfert plus rapide des IgM en IgG et un déclin dans le titre global des anticorps produits. Les réactions lymphocytaires *in vitro* au HPK après immunisation ont été réduites parmi le groupe des myélomes et avaient tendance à baisser avec le temps, parallèlement à la chute de la concentration des anticorps sériques. La majorité des malades souffrant de myélome ont présenté une cutiréaction retardée à HPK, mais ces réactions étaient moins marquées que chez les sujets témoins. Chez les mêmes malades, on notait également une diminution de la réaction lymphocytaire *in vitro* à la streptolysine O et à la vaccine. Quant à la fonction immunitaire des malades souffrant de plasmocytomes, elle était semblable à celle des témoins.

L'immunité, tant humorale que cellulaire, en réponse à un antigène récemment découvert, la HPK, est troublée chez les malades souffrant de myélome multiple.

"Complications" of the Landry-Guillain-Barré-Strohl syndrome

E. G. KING, M.D., F.R.C.P.[C] and HAROLD JACOBS, M.B., M.MED.(CAPETOWN), M.R.C.P.(EDIN.), F.R.C.P.[C], *Edmonton, Alta.*

Summary: Prognosis for life in the Landry-Guillain-Barré-Strohl syndrome is dependent upon the development of respiratory and non-respiratory "complications" and their successful management. Review of the literature, a case history, and a study of 14 patients with this syndrome at the University Hospital, Edmonton, indicate that "complications" can be anticipated in virtually all areas of acute care management, including respiratory, gastrointestinal, urinary tract, central and autonomic nervous systems, metabolic, cardiovascular, and infectious disease. The proper management of patients with the Landry-Guillain-Barré-Strohl syndrome demands an awareness of the totality of care required and the presence of a hospital system that provides for vital system monitoring and support, and for ready interdisciplinary consultation.

The Landry-Guillain-Barré-Strohl (L.G.B.S.) syndrome is a relatively uncommon, acute polyradiculopathy characterized by (1) symmetric, flaccid, usually incomplete paralysis which may involve facial and bulbar musculature; (2) subjective sensory symptoms and less often sensory loss; (3) elevated cerebrospinal fluid protein concentration without a proportional rise in cell count; (4) normal body temperature, sedimentation rate and leukocyte count (except when elevations in these are produced by antecedent illness or "complications"); and (5) complete remission (if "complications" do not occur).[1] Although debate exists over etiology and pathogenesis, the disease sequence and pathology are consistent with the hypothesis that the L.G.B.S. syndrome represents a non-specific pial vascular response to an antigen-antibody reaction[1-4] elicited by a wide variety of infectious and toxic insults.[5] This results in leptomeningeal hyperemia and edema with nerve root compression at bony exits and subsequent myelin-axonal degeneration.[6] Clinically, the differentiation from post-infectious encephalomyelitis may be difficult.[7]

Although mortality rates of up to 66% have been described,[8] most recent series report death in from 2%[9] to 19%[7] of patients with the L.G.B.S. syndrome. Most deaths are from respiratory "complications". Ravn,[2] in reviewing 425 cases from the literature, found an overall mortality of 22%. It is of interest that the mortality appears to be somewhat less in children[10] and has been reduced in all series since the experience with acute respiratory support gained in the 1952-53 polio epidemics.[1, 2] Prognosis for life in the L.G.B.S. syndrome would appear to be totally dependent upon the development of "complications", both respiratory and non-respiratory, and their successful management.

Neuromuscular respiratory insufficiency and aspiration pneumo-

E. G. KING, M.D., F.R.C.P.[C], Assistant Professor, Department of Medicine, and Director, Intensive Care Unit, University of Alberta Hospital, Edmonton, Alberta. HAROLD JACOBS, M.B., M.MED.(CAPETOWN), M.R.C.P.(EDIN.), F.R.C.P.[C], Assistant Clinical Professor, Faculty of Medicine, University of Alberta, Edmonton, Alberta.

Reprint requests to: Dr. Harold Jacobs, Suite 560, Physicians' and Surgeons' Building, 8409-112th Street, Edmonton, Alberta.