Europ. J. Cancer, Vol. 15, pp. 163–171

0014-2964/79/0201-0163 $02.00/0

[^{125}I] Clq-Binding Activity and its Relationship with Anti-Epstein–Barr Virus Antibodies in Sera from Nasopharyngeal Carcinoma Patients*

J. P. LAMELIN,† C. VINCENT,‡ T. SOUISSI§¶ and J. P. REVILLARD‡

†*Unit of Biological Carcinogenesis, International Agency for Research on Cancer*, 150, *cours Albert Thomas*, 69372 *Lyon Cédex* 2, *France*, ‡*Immunology Laboratory, INSERM U*80, *Edouard Herriot Hospital*, 69374 *Lyon Cédex* 2, *France and* §*Salah Azaiz Institute, Bab Saadoun, Tunis, Tunisia*

Abstract—*The* [^{125}I] *Clq-binding activity* (*Clq BA*) *and the antibody titre against Epstein–Barr* (*EB*) *viral capsid antigen* (*VCA*), *EB nuclear antigen* (*EBNA*) *and early antigen* (*EA*) *were determined in sera from* 28 *nasopharyngeal carcinoma patients and* 66 *matched controls from Tunisia. The geometric mean titres* (*GMT*) *of anti-VCA IgG and anti-EBNA, as well as the prevalence of anti-EA, antibodies were significantly higher among the patients than in the control group. The increased Clq BA of NPC sera, as compared to that of control sera, is likely to reflect the presence of soluble immune complexes, to which the contribution of the various autoantibodies found in this disease seems to be negligible.*

In individual sera from these patients, the Clq BA correlated positively with the titre of IgG ($P=0.026$), *but not with that of IgA, anti-VCA antibodies, indicating that the age-dependency of these different parameters was not* per se *a sufficient condition for a positive correlation to appear. Since the correlation between Clq BA and anti-VCA titre was restricted to the class of Ig which binds Clq, an alternative explanation is proposed in which specific anti-EBV antibodies complexed to virion structural antigens account for part of the elevated Clq BA.*

INTRODUCTION

SERA collected from patients with a large variety of diseases have been screened during these recent years for the presence of circulating immune complexes using one of the eighteen techniques presently available [1]. Their presence in sera from tumour bearing animals [2, 3] and cancer patients [4–6] has been reported by several investigators and an interesting hypothesis raised about their possible interference with some of the cytotoxic mechanisms [7] susceptible, as suggested by different *in vitro* models, to control the tumour growth *in vivo*.

Heimer and Klein [8] recently reported that sera from the two Epstein–Barr virus-(EBV-) associated tumours (Burkitt's lymphoma-BL, and nasopharyngeal carcinoma -NPC) contained circulating immune complexes, as shown by the ability of their serum to fix complement in a macroassay and to give "positive" results in the Raji cell binding assay [9].

Elevated titres of anti-EBV antibodies of both IgG and IgA classes constitute one of the hallmarks of NPC. This increase may represent, as does the presence of autoantibodies, one manifestation of the immune disorders possibly responsible for the increased tumour susceptibility [10]. It may also result from an unusually high and sustained antigenic stimulation, as suggested by the recent finding that malignant epithelial cells from NPC tumours may, under certain conditions, produce and shed structural EB virion antigens [11], a situation known to occur with other herpesviruses [12]. As a consequence of

Accepted 26 June 1978.

*Supported in part under contract No. 1 CP43296 within the Virus Cancer Programme of the National Cancer Institute (USA). Part of this work was presented at the 26th Colloquium on "Protides of the Biological Fluids", Brussels, Belgium, May 1978.

¶Present address: Blood Bank, University Hospital, Sfax, Tunisia.

this release, EBV-specific immune complexes are likely to be formed and found within the circulation. A similar situation has been documented in other pathological conditions such as, for example, those following hepatitis B virus infection [13].

We now report, using the Clq-binding assay of Nydegger *et al.* [14] that the sera from 28 Tunisian NPC patients have a [^{125}I] Clq-binding activity (Clq BA) greater than that of matched control sera. Furthermore, a positive correlation exists, in individual sera, between the level of Clq BA and the anti-VCA IgG antibody titres, suggesting that EBV-related antigens associated with their specific antibodies might account for at least part of the Clq-binding.

MATERIALS AND METHODS

Patients

Twenty-eight patients with biopsy-proven NPC and 66 controls matched for their ethnic origin were bled in Tunis (Salah Asaiz Institute) and their sera sent to Lyons (France). The sex and age distributions of patients and controls appear on Fig. 1 or Fig. 3. All sera were kept at −70°C, without preservative. None was frozen and thawed more than twice. All tissue sections were examined by the same pathologist (Dr M. Cammoun) and characterized as NPC according to criteria previously described [15]. Staging refers to the classification proposed by Ho [16]. No attempt was made to assess the presence of EB nuclear antigen (EBNA) or viral genome within the malignant cells.

EBV serology

Antibodies against viral capsid antigen (VCA) and early antigen (EA, R+D) were titrated by the indirect immunofluorescent technique, as described by Henle [17, 18]. The fluorescein-conjugated goat anti-human IgG antibody was obtained through the National Cancer Institute of the United States, from the Huntingdon Research Center (lot ref. 1126501). The failure of the fluorescein-conjugated anti-human IgA antibody (Hyland Laboratories, Costa Mesa California USA) to stain human myeloma cells containing γ, μ, λ or κ chains, confirmed its strict anti-α specificity. The anticomplement immunofluorescent (IF) test of Reedman and Klein [19] was used to determine the activity against EBNA.

Clq-binding assay

The technique of Nydegger *et al.* [14] was used with minor modifications as described by Zubler *et al.* [20].

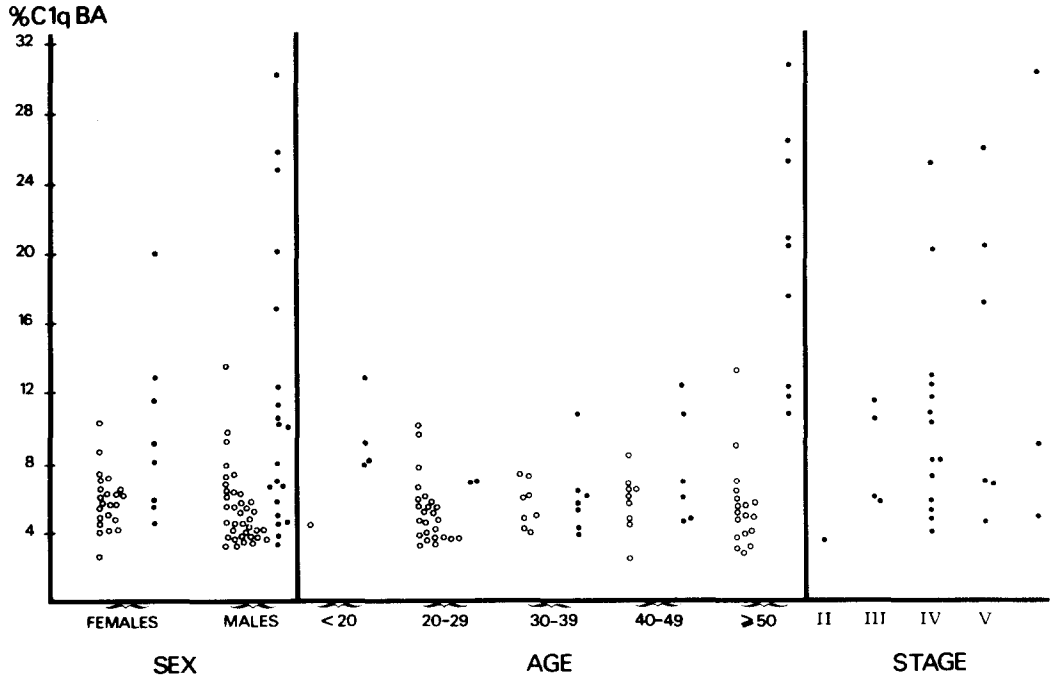

Fig. 1. Sex, age and stage distributions of Clq BA among NPC patients (black circles) and controls (white circles). In three cases (extreme right), no precise evaluation of the stage could be obtained.

RESULTS

Clq-binding activity (BA) of sera from NPC patients and controls

The respective influence of sex, age and stage on the Clq-binding capacity of NPC sera is illustrated on Fig. 1 where the age factor appears to be important, all patients over 50 having a Clq BA greater than 10%. No age-dependency was noted in the control group. Although 5 of the highest Clq-binding values belonged to stages IV and V, no significant association could be detected between NPC development and level of Clq BA (Fig. 1).

Clq-binding may be considered as a continuous or dichotomous variable. When all sera from either patients or controls were used to calculate the mean values, a difference was found (11.0 vs 5.5 respectively) between the two groups (Fig. 2). The unique distribution

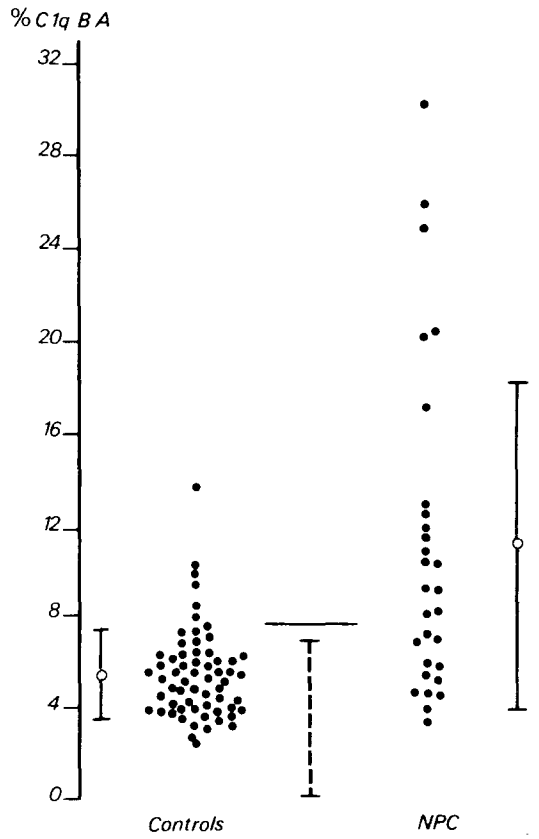

Fig. 2. Clq BA of sera from NPC patients and controls. *Black circles are values from individual sera. White circles correspond to the mean value of each group (±1 S.D.). The vertical dotted line gives the 95% confidence limits of a group of 100 Caucasians. The horizontal bar corresponds to the 90th percentile of the Tunisian control group.*

*Each serum, assessed for the presence of autoantibodies against actin (30), nuclear antigen (23) and lymphocyte membrane structures (31), was classified according to the number of "positive" assays (0–3).

however (S.D.: 7.0 and 1.9 respectively) prevented the use of Student's *t*-test for evaluating its statistical significance. The Clq-binding was therefore treated as a dichotomous parameter allowing to define "positive" and "negative" sera. Using as cut-off points either the upper limit of the 95% confidence interval of one hundred normal Caucasian sera (6.85%) or the 90th percentile of the Tunisian controls (7.5%), there were more positive sera in the group of NPC than in the control groups. The difference was statistically significant for either cut-off ($\chi^2 = 22.7$ and 25.7, respectively; $P < 0.001$).

EBV serology in sera from NPC patients and controls

The titres of IgG anti-VCA antibodies (GMT = 761) and anti-EBNA antibodies (GMT = 160 as well as the proportion of anti-EA positive sera (22/28) were higher in the group of NPC patients than in the control group (VCA = 115; EBNA = 85; EA = 21/65). All these differences are statistically significant (Table 1).

The anti-VCA titres in sera from NPC patients increased with age for both IgG and IgA classes (Fig. 3). There was no evidence of stage dependency in this small series.

Relationship between Clq BA and anti-EBV humoral immune response

The search for an association, in individual sera, between the percentage of bound Clq and the titre of anti-EBV antibodies resulted in the finding of a positive correlation for IgG anti-VCA ($r = 0.37$, $P = 0.026$) (Fig.4) but neither for IgA anti-VCA ($r = 0.097$; not significant) (Fig. 5), nor for anti-EA ($r = 0.30$, $P > 0.10$; not shown), nor for anti-EBNA ($r = 0.0048$, not significant, not shown).

DISCUSSION

We have found that sera from a series of 28 Tunisian NPC patients differed from that of controls by the higher frequency with which they bind radiolabled Clq at an abnormally high level. This increase is believed not to be artefactual but to reflect the presence of soluble immune complexes as shown by the following controls: 1. A series of Caucasian sera were repeatedly frozen and thawed, a small aliquot being collected before each freezing. Five sequential samples were assayed for their Clq BA. The level of binding, for a given serum, varied by no more than 20%

Table 1. Anti-EBV antibodies in sera from NPC patients and controls

	NPC (28)*	Controls (66)	Statistical analysis of the difference
VCA (IgG GMT)†	761	115	t test $P<0.001$
EBNA (GMT)	160	85	t test $P<0.05$
EA (Prevalence)	22/28	21/65	$\chi^2=16.85$ $P<0.001$

*Number of sera tested between parentheses.
†G.M.T. = Geometric Mean Titre.

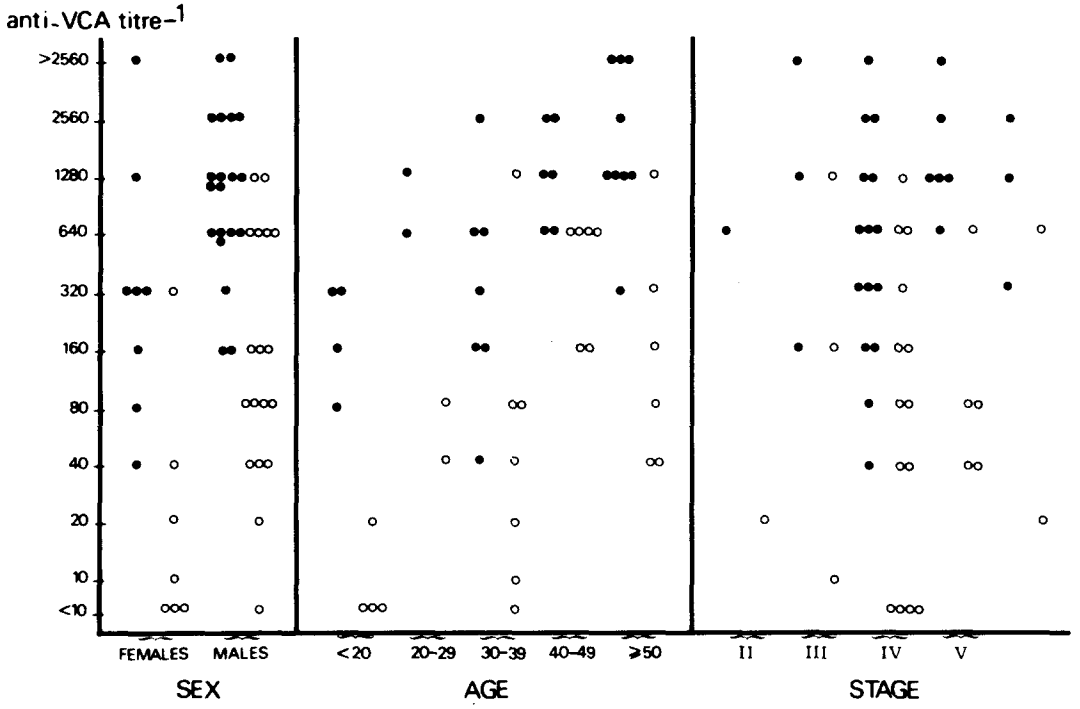

Fig. 3. Sex, age and stage distributions of anti-VCA IgG (black circles) and IgA (white circles) antibodies. In three cases (extreme right) information on the stage was lacking.

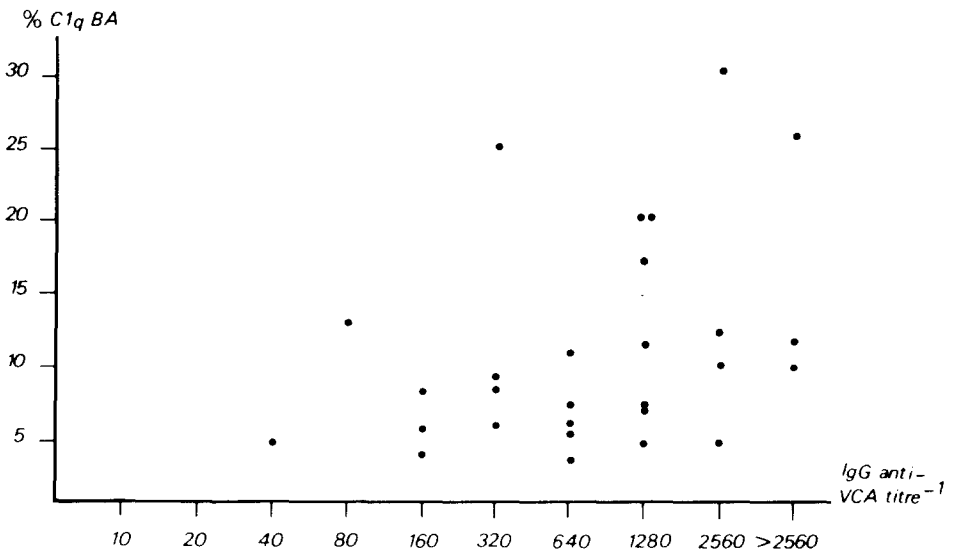

Fig. 4. Clq BA and anti-VCA IgG titre of individual sera from NPC patients. Correlation coefficient: $r=0.37$, $P=0.026$.

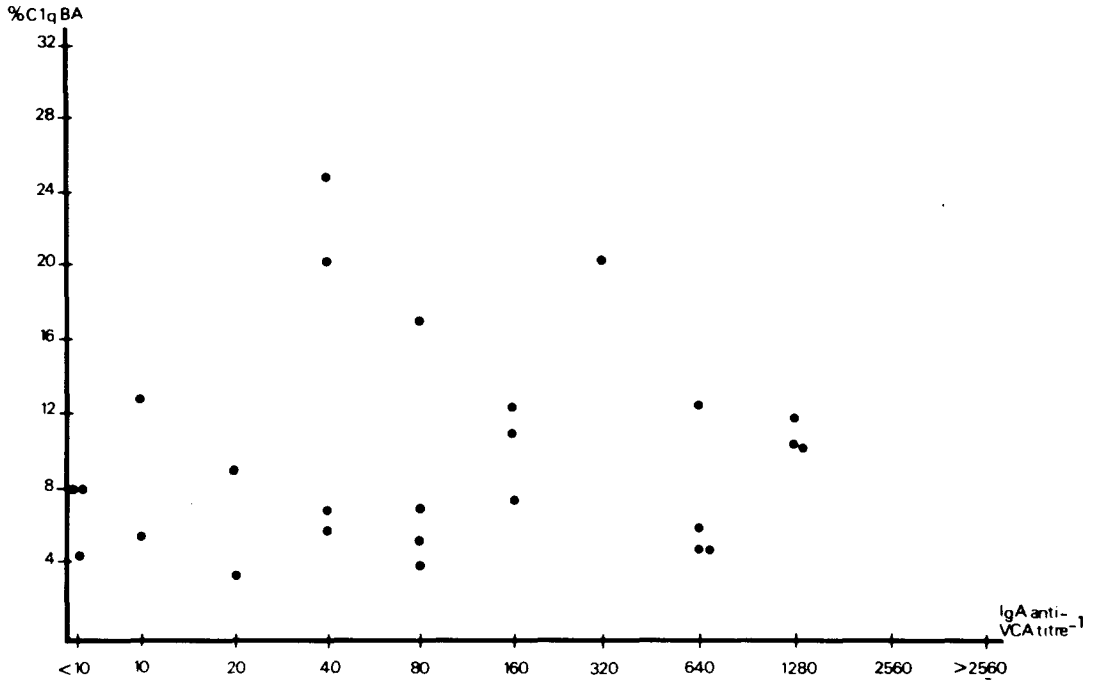

Fig. 5. Clq BA and anti-VCA IgA titre of individual sera from NPC patients. Correlation coefficient: $r=0.097$, not significant.

between the different aliquots (frozen from 1 up to 5 times), without any definite trend [21]. 2. The presence of anti-nuclear antibodies has been reported [22, 23] in sera from NPC patients. In view of the possible interference between Clq and circulating DNA [14] up to 500 μg/ml of native or denatured DNA were added to several human sera and found rather to decrease their Clq-binding capacity [21]. 3. No change in Clq BA was observed following the addition of lipopolysaccharides from *E. coli* (200 μg/ml) suggesting that bacterial contamination of sera during shipment was not a matter of concern. A more direct evidence for that was provided by the "normal" level of Clq-binding observed with the similarly handled control sera. 4. When a small series of sera with a high Clq BA (related to various pathological conditions such as systemic lupus erythematosus (SLE), cryoglobulinemia and chronic active hepatitis) were submitted to differential centrifugation, the Clq BA was recovered in the 15–21S region of the gradient, corresponding to molecular weights compatible with that of complexed 7S IgG molecules. 5. Finally, in view of the possible contribution of EBV-specific immune complexes to the increased Clq BA (see below), the possibility that EBV particles or fragments might bind Clq directly was explored, but neither the cell sap, nor the supernatant of the EBV-producing P3HR-1 cell line were found to bind Clq within the range of dilutions tested.

Our results confirm and extend the report, by Heimer and Klein [8], that in NPC (as well as in BL, the second malignant disease associated with EBV), a complement fixation macroassay and the Raji cell binding assay, gave "positive" results with 54 and 50% of the sera, respectively. Their use, however, of normal Caucasian sera as control group, made questionable the interpretation of their results, since the geographic origin of the specimens may by itself have influenced the level of circulating immune complexes [24, 25]. This is at least our experience from a limited survey of 15 BL cases and 15 matched controls from Uganda, in whom the mean values of Clq BA were similarly abnormal in the two groups (20.4% vs 23.7%).

In the present study, the Clq BA of sera from controls matched for age, sex and ethnic origin, were within "normal" limits, suggesting that the high Clq BA observed in sera from NPC patients was related to the disease. An interesting observation was the influence of age, the patients older than 50 having a much higher increase of their Clq binding level than that of patients from other age groups. the moderate increase observed with the latter fits with the absence of gross alterations of serum complement levels and contrasts with the high values observed in SLE, cryoglubulinemia and chronic active hepatitis (Vincent and Revillard, in press). From these results, however, the actual level of circulating antigen–

antibody complexes cannot be inferred since Clq does not bind to the Fc portion of some Ig, such as those from the IgA class, among which part of the anti-EBV antibodies are characteristically found in NPC patients [26, 27]. As to the lack of correlation between the level of Clq BA and the stage of the disease, it suggests that the tumour burden is a less critical parameter in determining that level than some age-dependent factor, possibly related to the well-documented alteration with age of several T cell functions [28] including the suppressor [29].

One of the main questions raised by the finding of soluble immune complexes is the specificities of the complexed antibodies. The presence of several autoantibodies has recently been reported in the serum from NPC patients: anti-nuclear factors [22, 23], anti-smooth muscle antibodies [30] and antibodies directed against lymphocyte cell surface determinants [31]. Whether they play a role in the Clq-binding increased activity of NPC sera, as anti-lymphocyte antibodies do in chronic lymphocytic leukemia [32], was examined by calculating the mean Clq-binding level in the four groups of patients classified according to the number, in individual sera, of the above autoantibodies (zero to three). As shown on Fig. 6, no difference was observed, suggesting that their contribution to the complexes, if any, is rather small.

Another obvious field of investigation was the humoral immune response to EBV, a

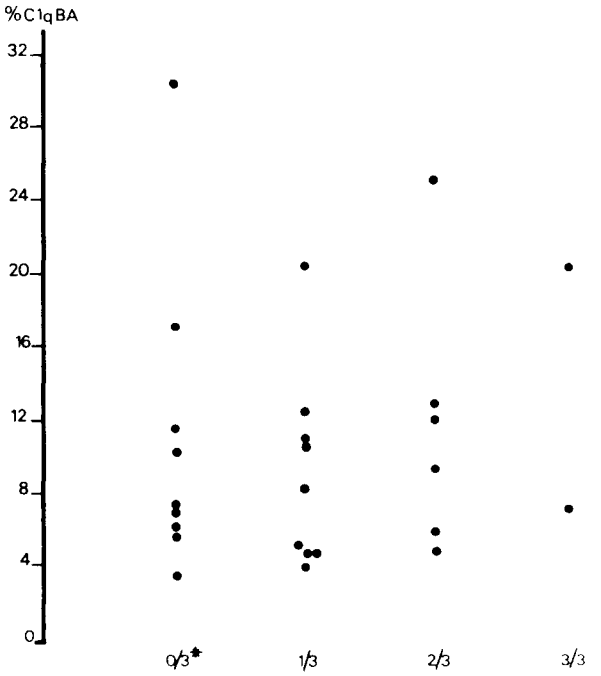

Fig. 6. Level of Clq BA in patients classified according to the number of different autoantibodies detected in their serum.

matter of extensive studies in NPC patients (reviewed in [33]). As known for a long time, high titers of antibodies against VCA, EA and EBNA are markers for NPC. This was once more confirmed in this small series of patients (Fig. 3).

The main finding, however, came from the search for correlations, in individual sera, between the level of Clq BA and the titres of the various anti-EBV antibodies. The positive correlation found with anti-VCA IgG antibodies raises several questions. First, as the titration of anti-VCA antibodies relies upon an indirect IF technique, it was essential to discard the possibility that immune complexes might be responsible for a technical "artefact". This was evaluated by calculating the GMT of anti-VCA antibodies in two groups of 40 sera from SLE, cryoglobulinemia and chronic active hepatitis, selected for their high (mean value: 34%; range: 10.4–79.8) or low (mean value: 3.9%; range: 1.9–6.2) level of Clq BA. No difference could be found (132 vs 136, respectively), indicating that the level of Clq binding *per se* was not responsible for the increased anti-VCA titre.

Another possibility was that an identical concentration of anti-VCA antibodies might have resulted in different titres depending upon their free or complexed status. Bridging of several IgG molecules through circulating antigens might well result in an increased avidity, for the virion antigen, of the complexed antibody as compared to the affinity of the unbridged molecule, a situation similar to the high avidity of IgM pentavalent molecules as compared to that of IgG bivalent molecules. This phenomenon could result in the binding of low affinity antibody to the EBV transformed test cells at dilutions which, with free antibody, would fail to give a positive staining reaction. Testing for this hypothesis is presently beyond technical possibilities.

Both Clq-binding level and anti-VCA titre being age-dependent, with their highest values in the patients over 50, the positive correlation between the two parameters may only reflect fortuitous dependency upon this third variable. It is tempting however to relate directly the two biological phenomena and to raise the hypothesis that part of the complexes detected in NPC sera are VCA-specific. Three lines of evidence suggest that this assumption may be correct. Firstly, the suspected antigens are structural virion proteins known to be present in saliva from NPC patients [27], to be produced by nude mice passaged tumour cells [11] and to elicit an increased specific

humoral immune response of both IgG and IgA classes [26, 27]. Secondly, although the highest anti-VCA titres were observed in our patients over 50 for both IgG and IgA, no correlation could be found between the latter and the level of Clq-binding, indicating that the age-dependency of both Clq BA levels and anti-VCA titres does not necessarily result in a correlation between the two biological parameters. Last, but not least, in 133 household contracts of these NPC patients, an identical correlation was found which could not be accounted for by this trivial explanation.*

In the alternative hypothesis of a specific involvement of anti-VCA antibodies in the immune complexes, the prediction can be made that a correlation is likely to exist between Clq-binding level and anti-VCA titres only for those antibodies which bind Clq. In addition, such a correlation is expected to be weak since, irrespective of any EBV association patients with tumours of many different types are known to have circulating immune complexes [5, 6]. Our finding that the weak correlation between Clq-binding level and anti-VCA titre is restricted to the IgG class supports these contentions. Secondly, immune complexes with VCA specificity were actually found by Oldstone *et al.* [4] in the kidney of two BL patients, the other EBV-associated tumour. The similarities, as far as the immune response to EBV is concerned, between NPC and BL, allow the reasonable assumption that immune complexes in both diseases may have similar specificities. Thirdly, a human situation already exists of a benign disease of viral etiology (acute viral hepatitis), giving rise to specific immune complexes [34], with long term prospect of malignancy (liver cancer) [13]. To decide whether this analogy is tenable requires first to identify the nature of the antigen present under complexed form in the sera from NPC patients, a work presently in progress in our laboratory.

*J. P. Lamelin, C. Vincent, B. Charnay, T. Soussi and J. P. Revillard. Anti-Epstein Barr virus antibodies, [^{125}I] Clq-binding and their relationship in sera from household contracts of nasopharyngeal carcinoma patients (submitted for publication).

Acknowledgements—The technical assistance of Ms. M. Flacher is gratefully acknowledged. We also thank Ms. C. Desgranges-Blanc, Ms. M. C. Favre-Beaut and Ms. M. F. Lavoué for the EBV serology, Dr. N. E. Day for advice with the statistics, Mr. J. Dechaux and Mr. P. Cattand for the pictorial and photographical work, and Dr. G. de-Thé for his continuous interest.

REFERENCES

1. P. H. Lambert, F. J. Dixon, R. H. Zubler, V. Agnello, C. Cambiaso, P. Casali, J. Clarke, J. S. Cowdery, F. C. McDuffie, F. C. Hay, I. C. M. MacLennan, P. Masson, H. J. Muller-Eberhard, K. Penttinen, M. Smith, G. Tappeiner A. N. Theofilopoulos and P. Verroust, A. collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. *New Engl. J. Med.* (In press).
2. R. W. Baldwin, M. R. Price and R. A. Robins, Blocking of lymphocyte-mediated cytotoxicity for rat hepatoma cells by tumor-specific antigen–antibody complexes. *Nature, New Biol.* **238,** 185 (1972).
3. M. B. A. Oldstone, Immune complexes in cancer: Demonstration of complexes in mice bearing neuroblastomas. *J. nat. Cancer Inst.* **54,** 223 (1975).
4. M. B. A. Oldstone, A. N. Theofilopoulos, P. Gunvén and G. Klein, Immune complexes associated with neoplasia: presence of Epstein–Barr virus antigen–antibody complexes in Burkitt's lymphoma. *Intervirology* **4,** 292 (1974).
5. R. D. Rossen, M. A. Reisberg, E. M. Hersh and J. U. Gutterman, The Clq-binding test for soluble immune complexes: clinical correlations obtained in patients with cancer. *J. nat. Cancer Inst.* **58,** 1205 (1977).
6. E. A. Samayoa, F. C. McDuffie, A. M. Nelson, V. L. W. Go, H. S. Luthra, and H. W. Brumfield, Immunoglobulin complexes in sera of patients with malignancy. *Int. J. Cancer* **19,** 12 (1977).
7. K. E. Hellstrom and I. Hellstrom, Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. *Advanc. Immunol.* **18,** 209 (1974).
8. R. Heimer and G. Klein, Circulating immune complexes in sera of patients with Burkitt's lymphoma and nasopharyngeal carcinoma. *Int. J. Cancer* **18,** 310 (1976).

9. A. N. Theofilopoulos, C. B. Wilson, V. A. Bokisch and F. J. Dixon, Binding of soluble immune complexes to human lymphoblastoid cells. II. Use of Raji cells to detect circulating immune complexes in animal and human sera. *J. exp. Med.*, **140,** 1230 (1974).
10. M. J. Simons, G. B. Wee, N. E. Day, S. H. Chan, K. Shanmugaratnam and G. De-Thé, Immunogenetic aspects of nasopharyngeal carcinoma (NPC)—IV. Probable identification of an HLA second antigen associated with a high risk for NPC. *Lancet* **i,** 142 (1975).
11. P. A. Trumper, M. A. Epstein, G. C. Giovanella and S. Finerty, Isolation of infectious EB virus from the epithelial tumor cells of nasopharyngeal carcinoma. *Int. J. Cancer* **20,** 655 (1977).
12. C. A. Daniels, S. G. Le Goff and A. L. Notkins, Shedding of infectious virus antibody complexes from vesicular lesions of patients with recurrent herpes labialis. *Lancet* **ii,** 524 (1975).
13. M. J. Simons, M. Yu and K. Shanmugaratnam, Immunodeficiency to hepatitis B virus infection and genetic susceptibility to development of hepatocellular carcinoma. *Ann. N.Y. Acad. Sci.* **259,** 181 (1975).
14. U. E. Nydegger, P. H. Lambert, H. Gerber and P. A. Miescher, Circulating immune complexes in the serum in systematic lupus erythematosus and in carriers of hepatitis B antigen. Quantification by binding to radiolabeled Clq. *J. clin. Invest.* **54,** 297 (1974).
15. M. Cammoun, G. Vogt Hoerner and N. Mourali, Tumours of the nasopharynx in Tunisia. An anatomic and clinical study based on 143 cases. *Cancer (Philad.)* **33,** 184 (1974).
16. J. H. C. Ho, The natural history and treatment of nasopharyngeal carcinoma. In *Oncology, Proceedings of the 10th International Cancer Congress.* Year Book Medical Publishers, Chicago. Vol. 4, No. 1 (1970).
17. G. Henle and W. Henle, Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bact.* **91,** 1248 (1966).
18. W. Henle, G. Henle, B. A. Zajac, G. Pearson, T. Waubke and M. Scriba, Differential activity of human sera with early antigens induced by Epstein-Barr virus. *Science* **169,** 188 (1970).
19. B. Reedman and G. Klein, Cellular localization of an Epstein–Barr virus (EBV) associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer* **11,** 499 (1973).
20. R. H. Zubler, G. Lange, P. H. Lambert and P. A. Miescher, Detection of immune complexes in unheated sera by a modified ^{125}I-Clq binding test. Effect of heating on the binding of Clq by immune complexes and application of the test to systemic lupus erythematosus. *J. Immunol.* **116,** 232 (1976).
21. C. Vincent and J. P. Revillard, Dosage de complexes immuns circulants. Comparaison entre l'inhibition d'agglutination et la précipitation de Clq en présence de polyéthylène glycol-EDTA. *Pathol. Biol.* **26,** 73 (1978).
22. T. O. Yoshida, High incidence of antinuclear antibodies in the sera of nasopharyngeal cancer patients. In *Recent Advances in Human Tumor Virology and Immunology.* (Edited by W. Nakahara, K. Nishioka, T. Hirayama and Y. Ito) Vol. 443. University of Tokyo Press, Tokyo (1971).
23. 23. J. P. Lamelin, J. P. Revillard, G. Gabbiani and G. De-Thé, Autoantibodies (cold lymphocytotoxins, anti-actin antibodies and anti-nuclear factors) in nasopharyngeal carcinoma patients. In *Nasopharyngeal Carcinoma-Etiology and Control.* (Edited by G. De-Thé and Y. Ito.) IARC Scientific Publication No. 18. International Agency for Research on Cancer, Lyon (In press).
24. W. H. O. Memorandum, Immunopathology of nephritis in Africa. *Bull. WHO* **46,** 387 (1972).
25. V. Houba, P. H. Lambert, A. Voller and M. A. O. Soyanwo, Clinical and experimental investigation of immune complexes in malaria. *Clin. Immunol. Immunopath,* **6,** 1 (1976).
26. G. Henle and W. Henle, Epstein–Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. *Int. J. Cancer* **17,** 1 (1976).
27. C. Desgranges, G. De-Thé, J. H. C. Ho and R. Ellouz, Neutralizing EBV-specific IgA in throat washings of nasopharyngeal carcinoma (NPC) patients. *Int. J. Cancer* **19,** 627 (1977).

28. J. P. GIRARD, M. PAYCHÈRE, M. CUEVAS and B. FERNANDES, Cell-mediated immunity in an ageing population. *Clin. exp. Immunol.* **27,** 85 (1977).
29. H. M. HALLGREN and E. J. YUNIS, Suppressor lymphocytes in young and aged humans. *J. Immunol.* **118,** 2004 (1977).
30. J. P. LAMELIN, E. H. WILLIAMS, T. SOUISSI, G. DeTHÉ and G. GABBIANI, Smooth muscle antibody in Burkitt's lymphoma and in nasopharyngeal carcinoma. *Clin. exp. Immunol.* **27,** 157 (1977).
31. J. P. LAMELIN, J. P. REVILLARD, J. M. CHALOPIN, J. H. C. Jo, T. SOUISSI, G. SCHWAAB and G. DE-THÉ, Cold lymphocytotoxic antibodies in nasopharyngeal carcinoma. *Brit. J. Cancer* **35,** 426 (1977).
32. N. K. DAY, J. B. WINFIELD, T. GEE, R. WINCHESTER and H. TESHIRA, Evidence for immune complexes involving anti-lymphocyte antibodies associated with hypocomplementemia in chronic lymphocytic leukemia. *Clin. exp. Immunol.* **26,** 189 (1976).
33. G. DE-THÉ, J. H. C. Ho and C. A. MUIR, Nasopharyngeal Carcinoma. In *Viral Infections of Humans: Epidemiology and Control.* (Edited by A. S. Evans) p. 539. Plenum Medical Book Corp., New York (1971).
34. A. M. PRINCE and C. TREPO, Role of immune complexes involving SH antigen in pathogenesis of chronic active hepatitis and polyarteritis nodosa. *Lancet* **i,** 1309 (1971).