

and 48S44.1 differed from one another in this aspect (fig. 1). Those 5 clones were further examined in the bioassay (table). Larvae fed with 10 times concentrated culture medium containing 5, 10, 20 and 40 ng toxin/larva, respectively, all exhibited 100% feeding inhibition (fig. 2, b). The same result was obtained with toxin and concentrated supernatants of clones derived from the 48S series (fig. 2, d). The only exception were the 4 larvae fed with supernatant of clone 48S54.1 and the lowest toxin concentration, showing a 80% feeding inhibition, i.e. a slight reduction in toxin activity.

In contrast, antibodies of clone 47S94.2 completely inactivated the toxin at toxin concentrations of 5 and 10 ng/larva (fig. 2, c). Increasing the toxin concentration to 20 and 40

ng/larva caused a weak feeding inhibition of 10 and 40%, respectively. Culture medium as well as all concentrated supernatants fed without toxin had no effect at all on the larvae (fig. 2, a).

We conclude that the antibodies described here detect non-repetitive, functionally different sites on the delta-endotoxin. 47S94.2 blocks the toxin activity. It is not yet clear whether inactivation of the toxin is due to a direct interference with the active site, an indirect effect as e.g. a conformational change of the antigen, or a blocking of its possible binding site.

Further studies on the characterization of the monoclonal antibodies and their mass-production in mice are in progress.

- 1 Reprint requests to P.L., Institute of Microbiology, Swiss Federal Institute of Technology, CH-8092 Zürich (Switzerland).
- 2 T.A. Angus, *Nature* 173, 545 (1954).
- 3 H.E. Huber and P. Lüthy, in: *Pathogenesis of invertebrate microbial diseases*, p.209. Ed. E.W. Davidson. Allenheld, Osmun, Totowa, N.J. 1981.
- 4 H.E. Huber, P. Lüthy, H.R. Ebersold and J.L. Cordier, *Archs Microbiol.* 129, 14 (1981).
- 5 F.P. Delafield, H.J. Somerville and S.C. Rittenberg, *J. Bact.* 96, 713 (1968).
- 6 M. Shulman, C.D. Wilde and G. Köhler, *Nature* 276, 296 (1978).
- 7 G. Galfre, S.C. Howe, C. Milstein, G.W. Butcher and J.C. Howard, *Nature* 266, 550 (1977).
- 8 G. Köhler and C. Milstein, *Nature* 256, 495 (1975).
- 9 J.W. Littlefield, *Science* 145, 709 (1964).
- 10 A. Voller, D.E. Bidwell and A. Bartlett, in: *Manual of clinical immunology*, p.506. Eds N. Rose and H. Friedman. American Society for Microbiology, Washington D.C. 1976.
- 11 H. Herbst and D.G. Braun, *Ann. Immun.* 132, 87 (1981).
- 12 R. Dulbecco and G. Freeman, *Virology* 8, 396 (1959).
- 13 R.G.H. Cotton, D.S. Secher and C. Milstein, *Eur. J. Immun.* 3, 135 (1973).
- 14 U.K. Laemmli, *Nature* 227, 680 (1970).
- 15 P. Lüthy, *Vjschr. naturf. Ges. Zürich* 120, 81 (1975).

Decrease of mast cells in regional lymph nodes in response to allogeneic antigens and syngeneic tumor antigens

K. Włodarski, S. Mazur and M. Jakóbsiak

Department of Histology and Embryology, and Department of Transplantation, Institute of Biostructure, Medical Academy, PL-02-004 Warsaw (Poland), 9 November 1981

Summary. The absolute number of mast cells in regional lymph nodes decreases on the 5th day after stimulation by allogeneic lymphocytes and semisyngeneic leukaemic cells, despite the enlargement of stimulated lymph nodes. It is postulated that the reaction of lymphatic mast cells could be a sensitive test for tissue incompatibility, and probably also for the presence of tumor associated antigens.

Basophilic granulocytes and mast cells (MC) play an active role in a variety of immune responses²⁻⁶ (for review see Włodarski et al.⁷) including antitumor reactions⁸⁻¹¹, but in spite of numerous experimental studies the mechanism and the significance of their participation remains to be elucidated¹².

MC rapidly respond by increase in number in murine lymph nodes regional to the site of a localized primary heterologous (BSA, sheep erythrocytes) antigenic stimulus^{3,4}, but within h this reaction ceases, and between 24 and 48 h the level of MC declines below the normal values⁴. According to the previous publication the early increase (within h) of the number of MC developing after injection of antigenic material, as well as of syngeneic cells seems not to be related to the antigenicity of the injected material⁴. In the present study an attempt was undertaken to find out what is the relation between the decline in the number of MC, following the early increase, and the antigenicity of the injected cells.

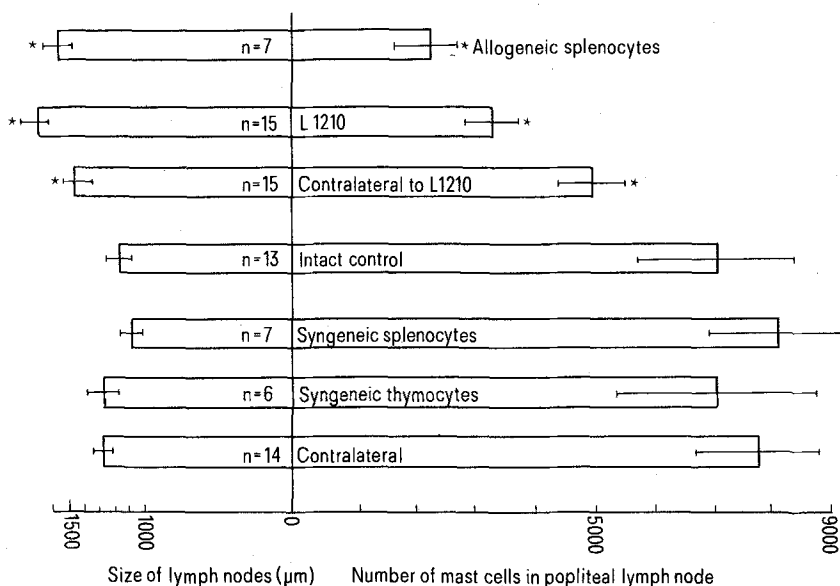
Material and methods. 4-5-month-old Balb/c × DBA/2WF₁ mice of both sexes, raised in our laboratory, were injected in the right footpad with 5 × 10⁶ semisyngeneic leukaemic L1210 cells, passaged on DBA/2W mice; with allogeneic splenocytes of strain 129; with semisyngeneic splenocytes and thymocytes of DBA/2W; or with Parker medium.

Contralateral left footpads were injected with 0.05 ml of Parker medium without serum. All cells were irradiated in vitro with 11000R, 3-4 h prior to the inoculation.

The animals were killed 5 days after injection and the popliteal lymph nodes were excised, fixed in Bouin solution and embedded in paraffin. The entire nodes were sectioned serially at 7 µm thickness. Sections were stained with 0.1% aqueous solution of toluidine blue. Every 5th section was carefully examined and the content of MC scored under the microscope at a magnification of × 120. From the number of sections obtained from a given lymph node and from the number of MC scored in every 5th section, the total content of MC per lymph node was calculated.

The size of a given lymph node, as expressed as its mean diameter, was calculated from the number of sections obtained multiplied by 7 µm. Arithmetical mean values for each group were calculated. The differences between mean values were analyzed by Student's t-test.

Results and discussion. The results obtained are presented in the figure. The MC number in the regional popliteal lymph node decreases after the injection of allogeneic splenocytes or semisyngeneic leukaemic cells, as compared to non-stimulated contralateral and intact control nodes. This difference is statistically significant as determined by Student's t-test at p < 0.05.



Changes of mast cell number and lymph node size in response to allogeneic antigens and semisyngeneic tumor antigens. Each bar represents the mean number \pm SE of mast cells (to the right of the line) and mean diameter and SE of lymph node stimulated by antigens indicated (to the left of the line). The number of specimens is given in each bar. Asterisks indicate the values different from that of intact control at $p < 0.05$.

Normal syngeneic cells did not cause any decrease in the number of MC in regional lymph nodes. Decrease in the number of MC following the injection of allogeneic lymphocytes or semisyngeneic leukaemic cells was accompanied by an enlargement of the stimulated lymph nodes ($p < 0.05$), clearly indicating that the reduction of MC was not due to a decline of lymph node cellularity.

Decrease of MC number is a very sensitive response of the

regional lymph node against antigenically alien cells as it is observed as a consequence of injection of both allogeneic cells as well as semisyngeneic leukaemic cells. Thus, this decrease is sensitive enough to detect tumor-specific antigens. It is worth mentioning that the number of MC in the stroma of human squamous cell cancer was 2–30 times lower than in the stroma of normal squamous epithelium or in the connective tissue distant from neoplastic epithelium¹³.

- Supported by the Polish Academy of Science, grant No. 10.5.04.3.
- J. J. Miller and L. J. Cole, *Nature* 217, 263 (1968).
- A. N. Roberts, *J. Immun.* 105, 187 (1970).
- K. Włodarski, N. M. Hancox, M. Zaleski and G. Zaleska, *Immunology* 24, 47 (1973).
- P. Keller, H. Cottier and M. W. Hess, *Immunology* 27, 1039 (1974).
- P. W. Askenase, *J. Allergy clin. Immun.* 64, 79 (1979).
- K. Włodarski, J. Włodarska and M. Zaleski, *Immun. polska* 2, 35 (1977).

- H. F. Dvorak, A. Dvorak and W. H. Churchill, *J. exp. Med.* 137, 751 (1977).
- H. M. Bowers, R. C. Mahaparto and J. W. Kennedy, *Cancer* 43, 569 (1979).
- W. R. Henderson, Chi Ey, E. C. Jong and S. J. Klebanoff, *J. exp. Med.* 153, 520 (1981).
- E. Farram and D. S. Nelson, *Cell Immun.* 55, 294 (1980).
- T. M. Dexter, R. W. Stoddort and S. T. A. Quazzaz, *Nature* 291, 110 (1981).
- K. Włodarski, A. Kukwa, O. Blaton, M. Dabska, R. Ruben, B. Borowiecki and A. Jeziorny, in preparation.

An H-2-associated difference in murine serum cholesterol levels

C. J. Meade and V. A. Gore

Lilly Research Center, Erl Wood Manor, Windlesham, Surrey GU20 6PH (England), 30 March 1982

Summary. We describe, in mice, a difference in serum cholesterol and adrenal weight associated with an H-2^a/H-2^b haplotype difference.

Genes of the major histocompatibility complex play an important role in determining patterns of immune response in both animals and man. Recent interest in immunological models of cardiovascular disease¹ has prompted a number of studies on the relationship between ischaemic heart disease and the presence of various histocompatibility antigens. Mathews² found a significant correlation between national death rates for ischaemic heart disease and population frequency of histocompatibility antigen HLA-8. However, other investigators, examining patients who had sustained a myocardial infarction, could find no significant increase in HLA-8 or any other histocompatibility antigen in this group^{3,4}. In neither of these patient studies were results stratified by serum cholesterol levels. In another

study⁵, which suggested a link between BW38 and premature coronary artery disease, the patient population was chosen to exclude hypercholesterolaemia.

Although heredity is known to exert a major influence on the development of hypercholesterolaemia, there has been done little work on the relationship of serum cholesterol levels and the major histocompatibility complex. Mathews, though observing a correlation between the geographical distribution of HLA-8 and higher population cholesterol levels, could not find a similar association with HLA-8 when investigating individual patients with hypercholesterolaemia⁶.

The mouse strains C57BL/10.ScSn (B10) and C57BL/10.A (B10.A) are genetically identical except in the major histo-