Ultrastructural Aspects of Antibody Plaque-Forming Cells from Clinically Normal and Overtly Autoimmune NZB Mice

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New Zealand Black (NZB) mice spontaneously develop autoimmune disease characterized by a high incidence, with age, of Coombs positive hemolytic anemia.\(^1,2\) NZB mice also differ quantitatively from "non-autoimmune" mouse strains in their responses to foreign antigenic stimuli. For example, Playfair\(^3\) and Evans, Williamson and Irvine\(^4\) have described an unusually early development of immune reactivity to sheep red blood cells (SRBC) in NZB mice, five- to seven-day-old animals showing near-adult levels of antibody formation. Morton and Siegel\(^2,5,6\) have observed this relative hyperactivity to SRBC also to pertain to six- to 18-week-old NZB mice. Elevated responses in young adult NZB mice have also been reported to such antigens as egg albumin and bovine gamma globulin,\(^7\) and to bovine serum albumin,\(^8\) accompanied by an inability to induce high-dose tolerance. In contrast, old overtly autoimmune NZB mice show depressed primary antibody formation to SRBC immunization\(^2,9,10\) and their spleen cells evidence diminished capacity to elicit graft-vs-host reactions in F1 hybrid recipients.\(^11\)

Electron microscopic studies have demonstrated murine leukemia type-C particles in the spleen, thymus, bone marrow, liver and kidneys of 10- to 12-month-old autoimmune mice of the NZB strain.\(^12\) Mellors and Huang\(^13\) have observed typical viruslike particles of the murine oncogenic type C to be present in the nephrons of NZB mice from birth until advanced age. These workers\(^13\) reported the induction in Swiss mice of hemolytic and renal disease by inoculation of newborn mice with cell-free preparations from type-C virus-containing splenic tissue of old overtly autoimmune NZB mice. The presence of murine leukemia viral particles in the tissues of NZB mice with characteristic autoimmune disease, but without symptoms of leukemia, and the production in Swiss mice of hemolytic and renal disease by inoculation of cell-free filtrates from splenic tissue of these mice have suggested a possible virus involvement of autoimmune disease.

East et al.\(^14\) observed autoimmune reactions and particles resembling murine leukemia virus in germ-free NZB mice, but suggested that much more experi-
mental evidence would be required before considering the association of virus-like particles, autoimmune disease and malignancy in this strain as established. In a more recent study, Prosser observed particles resembling murine leukemia virus in the organs of conventional NZB mice prior to birth and throughout life, indicating that these virus-like particles may have been present in their lymphoid tissues long before any autoimmune or neoplastic changes appeared. In this context, the relationship of the presence of such particles to the immunological behavior and autoimmune condition of NZB mice has not been clearly elucidated. The present study was directed to seeking morphological characteristics, including the presence of virus-like particles, of antibody plaque-forming cells from spleen cell suspensions from immunized NZB mice, in an effort to clarify such a possible relationship. For purposes of comparison with a nonautoimmune mouse strain, plaque-forming cells from young and old BALB/c mice were examined.

**Materials and Methods**

The NZB mice used as breeding stock were received as generations 57 and 58 from Mr. W. Hall, University of Otago Medical School, Dunedin, N.Z. BALB/c strain mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Response by these animals to SRBC was studied by the formation of hemolytic antibody plaques by spleen cells. A total of 78 NZB and 68 BALB/c mice of both sexes were studied. Of the former, 48 six- to eight-week-old and 30 Coombs positive nine- to 11-month-old mice were injected intraperitoneally with $1.5 \times 10^8$ saline-washed sheep red blood cells; 48 six- to eight-week-old and 20 10- to 14-month-old BALB/c mice were similarly injected. Animals were sacrificed three-seven days after immunization for spleen plaque assays.

For ultrastructural analysis, plaques from spleen cell suspensions from four-six animals of each of the various groups were collected on days four and five postimmunization from zones of hemolysis displaying only one distinct central antibody-forming cell. The cells were fixed at 4°C for at least two hours by layering the agar plates in which the plaques had developed with 1.5 per cent gluteraldehyde in 0.067 M cacodylate buffer, pH 7.4 containing one per cent sucrose. Employing an inverted microscope, the plaques were then removed from the agar plates with a capillary tube and placed in a buffer wash solution overnight. They were postfixed in osmium tetroxide, dehydrated with ethanol and embedded in Araldite. To facilitate subsequent orientation and sectioning of the cells, one drop of Richardson's stain was added to the 50 per cent ethanol in each vial at the beginning of the dehydration procedure. Subsequent dehydration removed most of the stain from the agar, but left the sheep red blood cells and the plaque-forming cells clearly stained. During sectioning, the distance from the block face to the cell was calculated by viewing the block face from above with a microscope having a calibrated fine adjustment. Thin sections were cut with a diamond knife and stained with lead citrate and uranyl acetate. Sections were viewed with a Philips EM 200 electron microscope operated at 60 kv., with a double-condenser lens system, anticontamination device, and a 50-μ objective aperture.

**Results and Discussion**

Spleen antibody plaque-forming cell responses of the young and old NZB and BALB/c strain mice which provided plaque-forming cells for electron microscopic examination are presented in Fig. 1. A somewhat-higher concentration of antibody-producing cells per mg. of spleen was observed in the case of old as compared to young-adult BALB/c mice. These differences were similar to the differences in primary antibody forming potential with age reported by Albright and Makinodan for (C3HX101)F1 and (C57B1XC3H)F1 hybrids.
Spleens of young NZB mice showed considerably more plaque-forming cells than either BALB/c group, while plaque formation by old NZB mice was markedly decreased. More detailed discussions of antibody plaque formation by NZB mice have been reported by us and others.\textsuperscript{2-6,9,10} Average spleen weights of the young and old BALB/c mice employed in the present study were 138 and 168 mg., respectively, and for the young and old NZB mice were, respectively, 161 and 451 mg.

Plaque-forming cells from young NZB mice had the appearance of plasma
Fig. 2.—Plaque-forming cell from young NZB mouse. Distinctive nucleus (N) contains masses of dense heterochromatin located peripherally as well as centrally. Less-dense nucleolus (NC) lies between heterochromatin and euchromatin. Cytoplasm characterized by profuse granular endoplasmic reticulum (GER), moderate numbers of mitochondria (M) and many free ribosomes. (× 19,200)

Fig. 3.—Plaque-forming cell from young NZB mouse. At higher magnification, profuse endoplasmic reticulum (GER) noted to contain homogenous material. Occasional viruslike particle (V) seen in reticulum cisternae, as in this case, or budding into cisternae from membranes, in other instances. (× 90,000)
cells (Figs. 2, 3). The eccentric, round or irregularly shaped nucleus was observed to contain masses of electron-dense heterochromatin located peripherally, as well as throughout the nucleus. Large nucleoli, less dense than the heterochromatin, appeared to be located randomly in the nucleus. In the cytoplasm, the granular endoplasmic reticulum was well developed, and the cisternae of the reticulum were usually distended. Free ribosomes were numerous, and mitochondria were present in moderate numbers. The Golgi apparatus was well developed but was not observable in every section. Cells from young BALB/c mice (Fig. 5) appeared highly comparable to cells of the young NZB strain mice.

Plaque-forming cells from old NZB mice appeared relatively similar to those from young NZB mice, with the exception of greater dilatation of the endoplasmic reticulum in the cells of older mice (Fig. 4). Some cells from the old Coombs positive NZB mice manifested increased fragility and a tendency towards early death as compared to those from young NZB mice. Cells from old BALB/c mice (Fig. 6) were generally similar to those of old NZB mice, but showed less endoplasmic reticulum dilatation, and tended to be less fragile with fewer indications of cell necrosis than their NZB counterparts.

Viruslike particles (Fig. 3) were seen located in the cisternae of the endoplasmic reticulum in sections from both young and old NZB mice. Parenthetically, similar type-A viruslike particles have been observed previously in spleen plaque-forming cells from BALB/c mice infected with Rauscher virus. As also

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**Fig. 4.—Plaque-forming cell from old, autoimmune NZB mouse.** Nucleus (N) of this antibody-producing cell from older NZB mouse similar to that seen in younger NZB mice. Cytoplasm shows cisternal distention of granular endoplasmic reticulum (GER). Small portion of Golgi apparatus (G) noted, as is nucleolus (NC). ($\times$ 17,400)
Fig. 5.—Plaque-forming cell of young BALB/c mouse. Cell shows large amount of granular endoplasmic reticulum (GER); perinuclear zone containing mitochondria (M); focus of small vesicles, possibly related to Golgi apparatus (arrow); occasional virus-like particles (V). Nucleus (N) also depicted. × 34,000

Fig. 6.—Plaque-forming cell of old BALB/c mouse. This cell, although reflecting morphological variability occurring among cells, shows nuclear (N) and cytoplasmic components typical of active antibody-producing cells. Centriole (C), microtubule (MT) and virus-like particles (V) noted. × 21,600
in the case of the control BALB/c mice (Figs. 5, 6), these viruslike particles appeared devoid of an electron dense nucleoid, and were not observed budding from plasma membranes. These latter observations were in contradistinction to the usual murine leukemia type-C viruses formed by processes of budding from the cell membrane into the extracellular space or into vesicles formed by the endoplasmic reticulum.\textsuperscript{12,22,23} The viruslike particles seen in plaque-forming cells of NZB mice would thus appear to be incomplete or noninfectious, and may not represent an agent responsible for immunologic aberration, either with reference to the development of autoimmune disease or to their response to foreign antigenic stimuli. Similarly, it is difficult to draw any correlation between the presence of viruslike particles or the overall morphology of these plaque-forming cells and the high incidence of malignant lymphoma and plasmacytoma in NZB animals.\textsuperscript{24} Further investigation, at the ultrastructural level, of the cells which are releasing autoreactive antibodies may contribute to the elucidation of the questioned association of viruslike particles, autoimmune disease and malignancy.

SUMMARY

Quantitative differences, in terms of antibody plaque-forming cells per mg. spleen, were observed in young and old animals of the BALB/c and NZB mouse strains. Ultrastructurally, antibody-producing cells from spleens of immunologically hyperactive young NZB mice appeared relatively similar to those from overtly autoimmune old NZB mice showing depressed immune responsiveness. There was more extensive dilatation of endoplasmic reticulum in cells of older mice, with some of these cells manifesting increased fragility. Viruslike particles, observed to be located intracisternally, lacked an electron dense nucleoid and were not seen budding from plasma membranes; they were thus unlike the murine leukemia type-C particles reported to be found in organs of NZB mice. Additionally, since these viruslike particles were observed in antibody-producing cells of young and old nonautoimmune BALB/c mice as well as in those of the NZB strain, it is probable that they do not represent the agent responsible for development of autoimmune disease or malignancy.

REFERENCES

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ANTIBODY PLAQUE-FORMING CELLS

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