Peptide Mapping Study of Anti-DNP-PLL Antibodies Produced by Guinea Pigs With and Without the PLL Gene* (32892)

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A single dominant autosomal gene determines whether or not guinea pigs can form antibodies to hapten-poly-L-lysine (PLL) conjugates (1,2). Animals lacking the gene, however, can be induced to form antibodies to haptens coupled to PLL if the hapten-PLL molecules are noncovalently complexed to negatively charged albumins which are themselves antigenic (3), but these guinea pigs do not display delayed hypersensitivity to hapten-PLL. Antihapten antibodies formed in this manner by guinea pigs lacking the gene are produced in similar amounts and have affinities of the same order of magnitude as the antihapten antibodies formed by responder guinea pigs immunized with hapten-PLL conjugates; both antibodies exhibit a similar degree of PLL specificity (3).

In an attempt to analyze whether the "PLL gene" codes for a unique segment in the variable portion of immunoglobulin chains in the antihapten-PLL antibodies formed by responder guinea pigs, peptide maps of the $F(ab')_2$ fragments of anti-2,4-dinitrophenyl-PLL (DNP-PLL) antibodies produced by responder guinea pig immunized with DNP-PLL and by guinea pigs lacking the PLL gene immunized with DNP-PLL • ovalbumin aggregates were compared and found to be almost identical.

Materials and Methods. Responder Hartley guinea pigs were injected with 0.1 mg of $DNP_{35}PLL_{430}$ in complete Freund's adjuvant in the four footpads (the subcripts refer to the average number of DNP and lysine groups per molecule), and 12 days later sera from 15 animals were collected and pooled. The PLL negative guinea pigs were immunized with 0.2 mg of $DNP_{35}PLL_{430}$ • ovalbumin as described (4), and after 18 days sera from 14 animals were collected and pooled. Anti-DNP antibodies were isolated from the two pools (responder and "non-responder") by the method of Farah *et al.* (5), and were digested with pepsin at pH 4.2 (6). When examined by the Ouchterlony technique, the $F(ab')_2$ fragments from the two pools gave a reaction of identity when tested with a rabbit antiserum against the $F(ab')_2$ fragment of nonspecific guinea pig γ_2 globulin, reactions of partial identity with intact γ_2 globulin with an antiserum against intact γ_2 globulin, and no reaction with an antiserum specific for the Fc-fragment of γ_1 globulin. For peptide mapping, $F(ab')_2$ fragments were extensively reduced with 0.3 *M* β-mercaptoethanol in 7 *M* guanidine • HCl (7), alkylated with iodoacetamide, dialyzed thoroughly against distilled water, and lyophilized. Maps of tryptic digests were then made according to the method of Katz *et al.*(8), as described previously (9).

Results and Discussion. Peptide maps of responder and "non-responder" anti-DNP-PLL $F(ab')_2$ fragments appear almost identical with the exception of 3 faint spots present in the responder maps (Fig. 1A and B). This conclusion was confirmed by maps of mixtures of equal amounts of the two preparations (Fig. 1C).

It can be concluded that, as far as can be determined with the technique of peptide mapping, the anti-DNP-PLL antibodies produced by PLL-positive and PLL-negative guinea pigs do not display significant structural differences in the portion of the molecules concerned with antibody specificity. (Whole antibodies, as opposed to $F(ab')_2$ fragments, were not used in order to avoid the large number of peptides that would be contributed by Fc fragments, which are not involved in specificity.)

Considering the known heterogeneity of the

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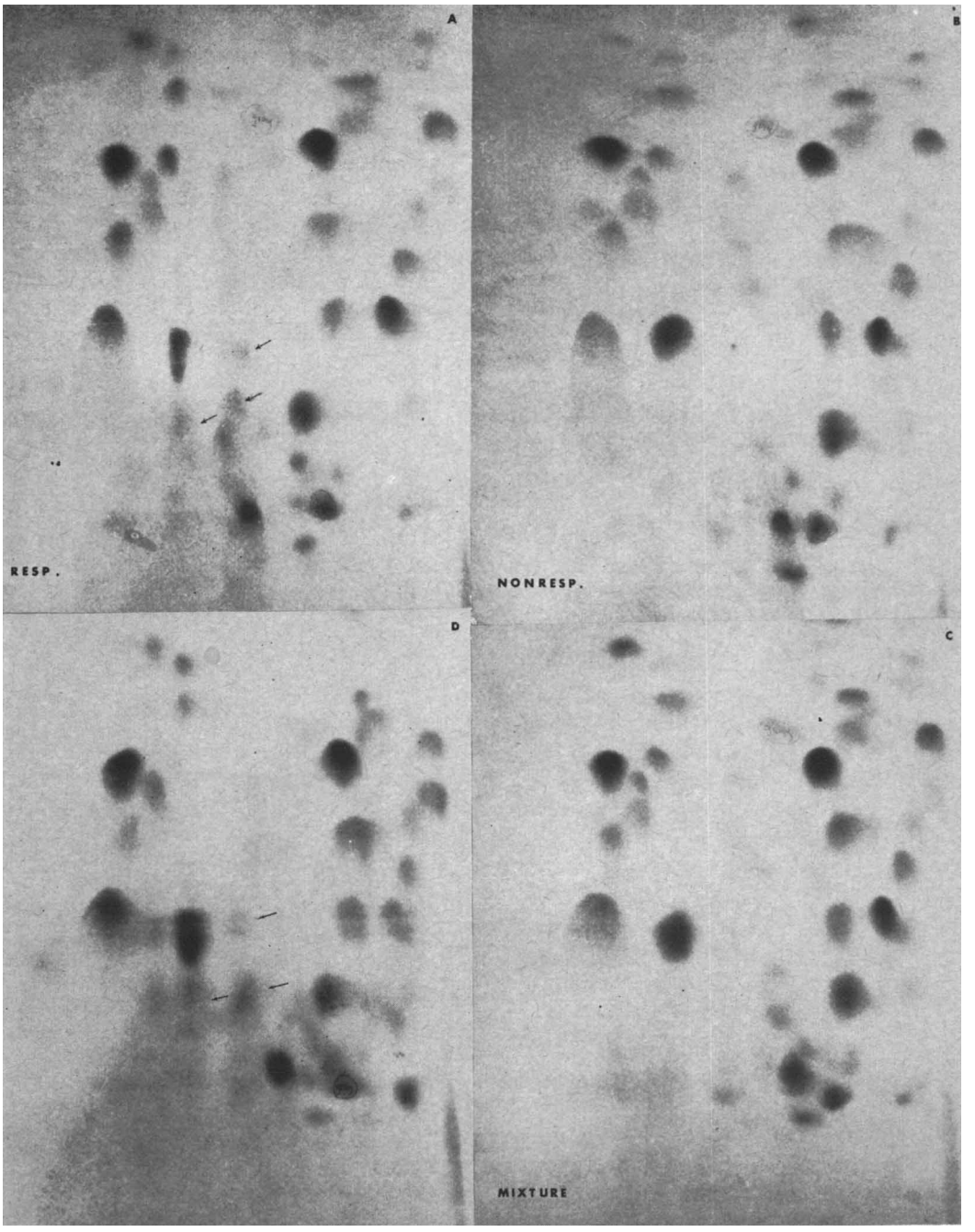

FIG. 1. Peptide maps of $F(ab')_2$ fragments from anti-DNP antibodies produced by responder (A) and "non-responder" (B) guinea pigs, and of a mixture of these preparations (C). The origin is at the lower right, chromatography is from right to left, and electrophoresis from bottom to top. The arrows denote the 3 faint spots present in the responder, but not in the "non-responder," maps. Three similar spots can be seen in a map of $F(ab')_2$ fragments from normal immunoglobulins and anti-DNP BGG antibodies (D).

antibody preparations studied, most, if not all, of the peptides observed come from the invariable portions of the L chains and Fd' fragments. The results obtained are, therefore, not surprising, but beforehand it was considered possible that if the "PLL gene," which is required for the immune response to DNP-PLL codes for some partial specificity for PLL, it could determine the sequence of a common segment in the variable portion of the L chain or the Fd fragment of anti-DNP-PLL antibodies produced by responder guinea pigs. Such a segment would be expected to be absent in antibodies produced by guinea pigs lacking the PLL gene. If such a structural difference did exist, it might result in extra peptides in the maps of antibodies from genetic responder animals. This result was not observed. The minor differences consisting of 3 faint spots present only in the maps of $F(ab')_2$ fragments from responder animals would seem to be insufficient to support this interpretation for the following reasons: the 3 spots in question were relatively faint and probably do not represent sequences common to all the molecules in the sample, and in other experiments similar spots were observed in maps of $F(ab')_2$ fragments from normal nonspecific immunoglobulins and from anti-DNP-bovine gamma globulin (BGG) antibodies (Fig. 1 D). Therefore, these experiments are consistent with the concept that the PLL gene does not control directly the strucure of anti-hapten-PLL antibodies but acts at an earlier step in the immune response, perhaps at the stage where antigen has to be specifically processed to be immunogenic.

Summary. Peptide map of $F(ab')_2$ fragments of anti-DNP-PLL antibodies obtained from responder guinea pigs immunized with DNP-PLL and from guinea pigs lacking the PLL gene immunized with DNP-PLL • ovalbumin complexes were compared. No structural differences that could be attributed to the "PLL gene" were observed.

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Biologic Activity of African Lymphoma Extracts* (32893)

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The present report describes certain biologic effects observed in cultured cells following treatment with extracts of malignant lymphomas (Burkitt's tumor) collected in Africa. The observations were made as part of a combined field (1) and laboratory study of the disease which is a common form of malignancy among children in central Africa (2) and New Guinea (3) and occurs infrequently elsewhere.

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Materials and Methods. Patients. The illnesses represented in the present study conformed to the pattern described by workers in Africa (4, 5) and the average age of the patients was seven years.

Specimens. Tumor biopsies were collected in the operating room where cytologic preparations were made and samples selected for histologic examination. Since many of these tumors contain large areas of necrosis, the