Occurrence of Ovine Homocytotropic Antibody[1] (34249)

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Recent investigations in several laboratories have suggested similarities in the class of antibody, designated as homocytoptropic antibody (HCA), which mediates local cutaneous hypersensititivity in the rabbit, man, monkey, and dog. For example, studies have indicated that dog, monkey, and rabbit HCA is a 2-mercaptoethanol labile immunoglobulin (Ig) of fast γ electrophoretic mobility, with a sedimentation coefficient of 7–9S (1–3). Furthermore, the frequency of occurrence, level and persistence of HCA for the rabbit appeared to be a function of the dosage and type of antigen and the adjuvant used for immunization (2).

The present investigation was designed to establish the occurrence and role of homocytotropic antibody in the spectrum of sheep Ig, and to determine the effect of antigen dosage and adjuvant on the synthesis of HCA in the antiprotein antibody response of sheep.

Materials and Methods. Five groups of three to seven sheep were immunized initially with 6, 30, or 60 mg of alum-precipitated human γ-globulin (AP-HGG) or with 6 mg of HGG in complete (CFA) or incomplete (IFA) Freund's adjuvant. All sheep of the AP-HGG groups were restimulated with 20 mg of AP-HGG at 90 days postprimary immunization. For the Freund's adjuvants groups one-half of each group was restimulated with 6 mg of AP-HGG and the remainder was restimulated with the primary immunogen and adjuvant.

Homocytotropic antibody was analyzed by a modified Prausnitz-Kustner (P-K) technique (2) in which intradermal sensitization was accomplished with 0.2 ml of whole serum. After a 48-hr latent period, 7.5 ml of 4% Evans blue were inoculated intravenously. Thirty min later, the skin was checked for nonspecific extravasation of dye and 10 μg of HGG in a volume of 0.1 ml was inoculated intradermally.

The Ig distribution of antibody activity was anlayzed by radioimmunoelectrophoresis (RIEP) to correlate the presence of HCA with antibody-binding activity (4).

Results and Discussion. Typical positive P-K reactions were generally 3–8 mm in diameter. The intensity of ovine dermal reactions was generally less than that observed for the rabbit, although the frequency of oc currence was as great, or greater.

The minimal latent period necessary to obtain specific, positive P-K reactions was 24 hr. At 48 hr, reactions were visible although slightly diminished, and at 96 hr were no longer detectable. Prior to 24 hr positive P-K tests could be observed but were obscured by nonspecific localization of dye.

The lack of intensity of the ovine reaction and relatively short latent period (5) may have been the result of either differences in ovine dermal sensitivity or to lower HCA titers in sheep or both.

The maximal HCA response was detected for the 6 mg AP-HGG group; reached a peak at 6–8-days postimmunization and declined thereafter (Table I). HCA was detected for all or most of the sera obtained during the initial phase of the antibody response.

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TABLE I. Ovine Homologous Skin Sensitizing Activity to HGG.

Primary dose (mg)	Postprimary (days)								Secondary dose (mg)	Postsecondary (days)			
	4	6	8	11	14	21	28	36[b]		4	8	15	21
6	0	7/7[a]	6/7	3/7	3/7	2/7	1/7	0	20	4/7	3/7	3/7	1/7
30	0	0	1/5	0	1/5	0	0	0	20	1/5	0	0	0
60	0	0	0	1/3	0	0	0	0	20	0	0	0	0

[a] Ratio of positive P-K reactions to total sera tested; O indicates nonspecific blueing less than 3 mm in diameter.

[b] No positive reactions were detected at 56 or 89 days postprimary immunization.

The high frequency of HCA was confirmed by a subsequent experiment in which HCA was detected for 4 of 5 of the sera obtained from sheep within 21 days postprimary immunization. Following secondary stimulation, only 4 of 7 sera from the initial low dosage (6 mg of AP-HGG) group had positive reactions. However, an anamnestic response for HCA was suggested by its earlier appearance in the secondary response. Only scattered positive reactions, in both the primary and secondary response, were detected for sera from the initial high dosage groups (30 and 60 mg of AP-HGG).

In contrast to the results after immunization with AP-HGG, no HCA was detected following primary stimulation with HGG in IFA or CFA. Subsequently, when one-half of the sheep of each Freund's adjuvant group was restimulated with 6 mg of AP-HGG no HCA was detected. However, it may be recalled, that this dosage of antigen had been sufficient to elicit effectively the primary synthesis of HCA.

The failure of high dosages of AP-HGG and low dosages of HGG in Freund's adjuvant to elicit HCA may have been due either to a failure to stimulate the cells synthesizing HCA or to the induction of tolerance in these cells. If the former were true the secondary stimulation with 6 mg of AP-HGG would have been expected to induce HCA synthesis. However, this was not observed. If the second hypothesis were true and tolerance was induced the sheep would be expected to have recovered their responsiveness after a sufficient interval of time.

To examine this hypothesis, the CFA and IFA sheep were given a tertiary stimulus of 6 mg of AP-HGG at 158 days following secondary stimulation. Four of eight sheep had HCA activity by day 7. A total of 5 of the 8 sheep tested had positive HCA reactions at sometime between days 7 and 21. Therefore, it appears that if tolerance was induced, it was lost in 5 of 8 sheep by day 158, a result not incompatible with tolerance data for other species.

Selected HCA positive sera were tested for heat stability. Treatment at 65° for 1 hr abolished their activity. The lability of HCA activity was also indicated by the loss of all HCA activity after storage for a few weeks at —20°. These results are similar to those observed for rabbit HCA (2). More extensive characterization of ovine HCA was not undertaken due to the extreme lability of the antibody activity. Recent observations have suggested, however, that the levels and stability of HCA antibody activity may vary, depending upon the antigen used for immunization. Experiments to enchance ovine HCA are in progress and may facilitate isolation and characterization of this ovine Ig.

The occurrence of other classes of ovine antibody in the primary response to low and high doses of AP-HGG and HGG in Freund's adjuvants was analyzed by RIEP to relate HCA to other recognized classes of Ig. The IgG_1 and IgM antibodies were detected concomitantly at days 6–8. The IgM was detected through day 14. The IgG_2 was detected most frequently in the high AP-HGG dosage group, but declined more rapidly than IgG_1. The IgA was detected at day 11 in the primary response and increased through day 35. Results for the IFA and CFA sheep were similar to the AP-HGG groups except for

the absence of IgA and a greater persistence of IgG_2. Since HCA activity reached a maximum by days 6–8 and declined rapidly thereafter, the occurrence of HCA did not correlate with the appearance or persistance of IgG_1, IgG_2, IgA, or IgM as detected by RIEP. These results suggested that a fifth, as yet undesignated class of ovine antibody is the carrier of HCA.

Summary. Homocytotropic antibody was detected in 80–100% of the ovine sera observed early in the primary response after immunization with 6 mg of the alum-precipitated form of human γ-globulin. This form of antigen, particularly in low dosages, appeared to result in a selective potentiation of ovine homocytotropic antibody as compared to sheep immunized with antigen plus complete or incomplete Freund's adjuvant. The failure to observe any direct correlation between the detection of IgG_1, IgG_2, and IgA, and IgM by radioimmunoelectrophoresis and the occurrence of homocytotropic antibody activity suggested that another or fifth class of ovine antibody was responsible for this functional property.

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