THE CHEMILUMINESCENCE OF OVINE NEUTROPHILS

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ABSTRACT

The development, structure and function of polymorphonuclear leucocytes (PMN) is reviewed and methods for determining neutrophil competence are discussed.

A technique, based on differential centrifugation and red blood cell lysis, is described for isolating neutrophils of 80 to 90% purity from ovine blood. A standardised, luminol-enhanced chemiluminescence (CL) assay was developed for ovine neutrophils using latex beads as the phagocytic stimulus and some conditions influencing the level of CL generated are described.

Normal sheep of similar age, housed under identical conditions and bled at approximately the same time on different days produced CL responses ranging from 386 to 3084 millivolts (mV). Animals sampled once daily over 5 days showed large fluctuations in CL values both between and within individuals. Furthermore, sheep bled at 4 hourly and 6 hourly intervals for 48 and 96 hrs respectively produced CL responses in a single individual with a range of 618 to 2946 mV. There was no evidence of periodicity in CL activity over the time periods examined.

Since peak CL responses showed such large variations between individuals, integrated CL values were also measured. Variations between and within individuals similar to those recorded by peak CL were seen in these results.

To examine the possible role of genetic differences in neutrophil function on the variability of CL, pairs of bovine monozygous twins were sampled. There was no correlation in CL response between genetically identical animals with the CL values from pairs of animals differing by as much as 2943 mV.

The effect of cortisol on PMN CL was assessed. Synthetic corticosteroid *in vivo* and *in vitro* did not increase the peak CL response from isolated neutrophils.

Profiles produced by recording CL against time were examined. Some cell isolates produced single peaked profiles while others gave a double peaked response. Single and double peaked profiles were recorded from the same donor at different times during a 24 hr period. Storage of the cells for prolonged periods sometimes resulted in an increase in the magnitude of the first peak possibly indicating an increase in the amount of more readily available myeloperoxidase (MPO). Prominent first peaks were still displayed after the cells were washed and resuspended in fresh media suggesting that the more readily available MPO was cell attached rather than truely extracellular.

Neutrophils from ceroid lipfuscinosis-affected sheep produced peak CL responses and CL profiles similar to those given by normal sheep. These results did not confirm the postulated myeloperoxidase deficiency of this condition.

It is concluded that ovine neutrophil CL is subject to large variations which cannot be controlled by standardising the cell isolation and CL analysis techniques. The assay is therefore unsuitable as a measure of neutrophil function where single samples are examined. Where there are consistant differences between individuals over a number of days, then CL may be of use when considered in conjunction with other tests of PMN function.

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