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Variant-Specific Surface Protein Switching in *Giardia lamblia*

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Surface antigen switching in *Giardia lamblia* was analyzed using monoclonal antibodies specific for two variant-specific surface proteins (VSPs). Two VSPs were detected on the surface of single trophozoites. Dual expression persisted for 13 h but disappeared at 36 h, as in other parasites that undergo surface antigenic variation.

The major surface antigens of *Giardia lamblia*, variant-specific surface proteins (VSPs), undergo antigenic variation, where one VSP expressed by a single organism is replaced by another antigenically distinct VSP (8, 9). How VSP switching occurs on the surface of the trophozoite is unknown. To determine if two VSPs can be found on the surface of single trophozoites and the nature of switching, monoclonal antibodies (MAb) specific for two VSPs expressed in the WB isolate (14) were used in direct immunofluorescence assays. MAb 5C1 (9), an MAb that recognizes VSP1267 (9), was directly conjugated to fluorescein (10); MAb 9B10, an MAb that reacts with the newly described VSP9B10, was conjugated to biotin (EZ-Link NHS-Biotin; Pierce, Rockford, Ill.) and subsequently reacted with phycoerythrin (PE) conjugated to avidin (Molecular Probes Inc., Eugene, Oreg.). This procedure allowed fluorescence-activated cell sorting (FACS) with a single laser and confirmation and subsequent analysis by direct fluorescence microscopy.

MAb 9B10 was produced as described earlier (6) and showed a pattern typical of VSP-reacting antibodies (11). Verification that MAb 9B10 reacted with a VSP was obtained by sequence analysis of multiple clones from cDNA λgt22a (6) and Uni-Zap XR (Stratagene, La Jolla, Calif.) expression libraries recognized by MAb 9B10. The Uni-Zap XR library was made from clones predominantly expressing VSP9B10. Sequences from the 5′-terminal coding and untranslated regions were determined by inverse PCR of genomic DNA from clones expressing VSP9B10 (3). Reacting clones yielded sequences coding for the same protein. The coding region was 2,220 bp long and predicted to encode a protein of 740 amino acids (GenBank accession number AY007596). Features characteristic of VSPs, including a conserved carboxyl terminus (7), multiple CXXC (1) and GGCY (12) motifs, and a modified Zn finger motif (8), were found in VSP9B10. MAb reactivity resided in the amino-terminal protein fragment encoded by nucleotides (nt) 39 to 422. Multiple antisense oligonucleotides derived from the sequence (JC109 [nt 342 to 369], JC111 [nt 448 to 428], JC266 [nt 146 to 124], and MM16 [specific to the conserved 3′ region of VSPs]) (7) reacted in Northern blottings with the predicted 2.2-kb transcript from a clone of mostly MAb 9B10-expressing trophozoites; this result indicated that the sequence obtained was specific for the VSP reacting with MAb 9B10 (data not shown).

Giardia parasites were maintained in vitro as previously described (5). Dually labeled parasites were routinely obtained as follows. WB/1267 (9), a predominantly VSP1267 (recognized by MAb 5C1)-expressing clone, was allowed to degenerate and express other VSPs. Almost all clones that express a predominant VSP express less of the initial VSP over time. Therefore, at the time of VSP switching, a single trophozoite from this clone would tend to express VSP1267, the initial VSP, and begin to express another VSP in its repertoire, in this case, VSP9B10. Trophozoites were incubated with 100 μl of a 1:30 dilution of MAb 5C1 conjugated to fluorescein and a 1:10 dilution of MAb 9B10 conjugated to biotin in 1% *Giardia* medium in phosphate-buffered saline (washing fluid). After 1 h at 4°C, the trophozoites were washed three times and reacted with a 1:1,000 dilution of avidin conjugated to PE for 15 min; then, they were washed as described above. Controls consisted of identical cultures exposed to no MAb, a 1:30 dilution of MAb 5C1 conjugated to fluorescein, or a 1:10 dilution of MAb 9B10 conjugated to biotin and treated as described above.

Initally, PE-labeled trophozoites were harvested by FACS, followed by a second round of sorting to harvest parasites showing dual expression. Sequential sorting separated adhering trophozoites expressing a single VSP. The relatively few cells that showed dual expression and that were harvested prevented further analysis by FACS. Cells were then collected in medium and divided into three parts. One portion contained one-half of the cells, which were immediately viewed by direct microscopy and scored for the expression of 9B10VSP, 1267VSP, both VSPs, or neither VSP. After overnight incubation, usually 13 h, and again at about 36 h, the organisms were examined again. Controls included untested cells to preclude the continued presence of antibody to VSP from the initial sorting and singly labeled trophozoites to control for carryover of fluorescence. No fluorescence was detected after 13 h in controls, consistent with the known time course of VSP shedding.

Between 100 and 200 sequential cells were scored. XF25

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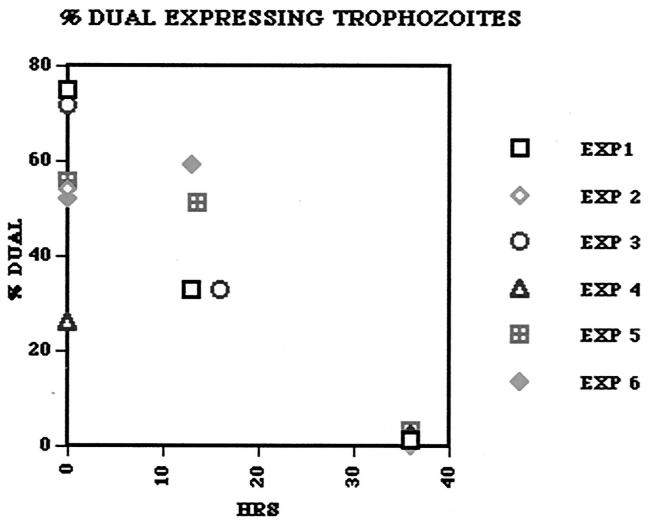

FIG. 1. Percentages of trophozoites showing dual expression over time.

(fluorescein) and XF34 (PE) fluorescence sets and a 535DF35 barrier filter were used to evaluate parasites showing dual fluorescence. Cells that were a definite yellow-orange and green, indicating the presence of both PE and fluorescein, cells that showed green fluorescence in the presence of the barrier filter, indicating fluorescein, and cells that were red-orange when the XF34 fluorescence set was used, indicating PE, were scored as showing dual expression. Such cells were easily identified. The intensity of staining of each VSP appeared fainter than that for trophozoites expressing a single VSP, suggesting that the number of molecules of each VSP on the surface of the trophozoites was smaller in cells showing dual expression. The percentages of cells showing dual expression decreased over time from 55.3% ± 17% (mean and standard deviation) at 0 h to 44.0% ± 13% at 13 to 16 h and then to 1.4% ±1.0% at 36 h (Fig. 1). In all experiments, the percentage of PE- or VSP9B10-stained cells increased over time compared to that of fluorescein- or MAb 5C1-stained cells. The percentages of cells showing PE staining in different experiments increased from 19, 49, 24, 32, and 40% at 0 h to 91, 79, 84, 55, and 82% at 36 h, respectively. These results are consistent with the observation that VSP1267-expressing clonal cultures tend to express other VSPs over time. In most experiments, only a few cells failed to express either of the two VSPs.

The variability of the decrease in expression over time among the experiments does not allow a firm conclusion to be drawn concerning whether the loss of surface expression is gradual or precipitous. The decrease in the percentage of trophozoites showing dual expression at 13 h was not significant compared to the results obtained at zero time. Nevertheless, with the assumption of linear decay ($y = -1.965x + 68.164$; $r = 0.972$), the half-life was calculated to be 17.3 h.

Dual expression was a transient process, suggesting that trophozoites showing dual expression were in the process of switching. In other experiments with different sets of MAb to other VSPs, dual expression was also observed, so that the phenomenon likely is common to all VSP switching. An exact measure of the percentages of trophozoites showing dual expression in cultures was difficult to accurately assess but was estimated to be on the order of 0.1 to 0.5% or 0.01 to 0.05%, usually in the lower range.

The failure to observe continued dual expression indicates that the expression of multiple VSPs on single trophozoites is rare or does not occur. It is typical for only one VSP to be expressed; when two are observed, the VSPs are in the process of switching.

Surface antigen switching in other protozoa that undergo antigenic variation has been studied. The presence of more than one variant surface antigen as well as the timing and duration of switching in both *Paramecium* (15) and *Trypanosoma* (2, 4, 13) are similar to those in *Giardia*. Two surface antigens were found on the surfaces of the two former parasites at the time of switching, and both showed the prolonged presence of both VSPs. Total disappearance occurred in 32 to 48 h in *Trypanosoma brucei rhodesiense* and in about 30 h in *Paramecium*, compared to 36 h or less in *Giardia*. Despite major differences in how and where these parasites live and obvious differences in the molecular mechanisms involved in antigenic variation, there appears to be a general similarity in the replacement of one surface antigen for another.

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