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Swine leukocyte antigen and macrophage marker expression on both African swine fever virus-infected and non-infected primary porcine macrophage cultures*

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ABSTRACT

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Swine leukocyte antigens (SLA) and a macrophage specific marker were monitored on porcine macrophages cultured with or without macrophage colony stimulatory factor (M-CSF) and on cells infected with African swine fever virus (ASFV). SLA expression was maximal either in the total cell extract or on the cell surface at 3–4 days of culture; after 4 days these values began to decrease. Fluorescence analyses of immunostained macrophages cultured with or without M-CSF indicated a major upward shift in the number of SLA Class I molecules on individual macrophages whereas for SLA Class II both a novel expression of Class II and an upward shift in the number of molecules per cell were evident.

Infection of 3-day-old macrophage cultures with three different isolates of ASFV resulted in minor changes in surface expression of SLA Class I, SLA Class II, and macrophage markers. No differences in infection with ASFV was observed whether macrophages were SLA Class II positive or negative, nor was there blocking by anti-SLA Class I or Class II monoclonal antibodies of ASFV infection of cultured macrophages.

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ABBREVIATIONS

APC, antigen presenting cell; ASF, African swine fever; ASFV, African swine fever virus; c.p.e., 50% cytopathic effect; DR-II, Dominican Republic II ASFV isolate; HAD_{50}, 50% hemadsorption units; LPS, lipopolysaccharide; L60, Lisbon 60 ASFV isolate; mAb, monoclonal antibody; M-CSF, macrophage colony stimulatory factor; MHC, major histocompatibility complex; NHV, non-hemadsorbing ASFV isolate; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; SLA, swine leukocyte antigen.

INTRODUCTION

Host resistance to viral disease depends in part on the appropriate recognition by the T-cell antigen receptor of both antigens, e.g. a viral peptide and either Class I or Class II antigens of the major histocompatibility complex (MHC) on the surface of the antigen presenting cell (APC) (Unanue, 1984; Bjorkman et al., 1988; Geppert and Lipsky, 1989). Reduced stimulation of T-cells may result from inadequate expression of either or both of these antigens, due to down-regulation of MHC antigen by viral infection of cells (Bernards et al., 1983; Jennings et al., 1985; Brown et al., 1988) or altered association of viral antigens with MHC antigens (Kane et al., 1989).

African swine fever (ASF) is caused by a large enveloped DNA virus of the family Iridoviridae (Breese and DeBoer, 1966) sharing many properties with poxvirus (Viñuela, 1987). African swine fever virus (ASFV) infects cells of the monocytic/phagocytic system (Hess, 1981) causing some functional alterations (Martins et al., 1988). The disease in domestic swine results in significant economic loss to the pig industry in endemic areas. Mortality in ASFV-infected pigs correlates with the virulence of the virus isolates (Pan and Hess, 1984) and occurs within 5–15 days of infection (Wardley et al., 1987). Although ASFV induces a strong antibody response, the antibody has no neutralizing activity and virus clearance mediated by antibody-specific mechanisms does not occur until after the peak of viremia is over. Thus, antibody does not seem to play a major role in determining survival of pigs infected with ASFV (Wardley et al., 1987). Specific immune mechanisms of ASFV clearance from infected pigs are poorly understood, hence, the prospects for development of vaccines are poor.

Studies of kinetics of the synthesis of the ASFV-induced polypeptides in Vero cells indicate two early and one late class of proteins (Santaren and Viñuela, 1986). Although chemical studies on ASFV antigen expression on the surface of infected Vero cells (Santaren and Vinuela, 1986; Revilla et al., 1988), infected IB-RS cells (Wardley et al., 1985) or infected porcine macrophages (Whyard et al., 1985) have been reported recently, the exact time course of ASFV antigen expression on the surface of infected cells is poorly characterized. We have reported changes in the expression of swine MHC or swine leukocyte antigens (SLA) in spleen tissue sections from animals infected with different isolates of ASFV (Gonzalez Juarrero et al., 1992). Since macrophages are important APC (Unanue, 1984), a decrease in SLA expres-

sion during ASFV infection of the porcine macrophage could limit the recognition of ASFV antigens by the immune system.

Long-term blood mononuclear cell cultures require the use of a supernatant from murine L929 cells as a growth factor, or macrophage colony stimulatory factor (M-CSF) (Genovesi et al., 1989). The present study analyzed SLA expression in both ASFV-infected and non-infected primary porcine macrophage cultures. Our objectives were to analyze the effects of M-CSF on the expression of SLA in factor-stimulated cultures and to characterize the effect of ASF viral replication on SLA expression.

MATERIALS AND METHODS

Animals and viruses

Yorkshire pigs, 6–12 months old, were conventionally maintained in the PIADC colony, and were bled from the ear into heparin (10 IU ml^{-1}) for these experiments. The highly virulent ASFV isolate, Lisbon 60 (L60) (Manso Ribiero and Rosa Azevedo, 1961), the moderately virulent isolate, Dominican Republic-II (DR-II) (Mebus, 1988) and non-hemadsorbing ASFV isolate (NHV) (Vigario et al., 1974) were grown on porcine macrophages. These three isolates replicate with similar kinetics when grown in vitro; their titer, expressed as 50% hemadsorption units (HAD_{50}) ml^{-1} or 50% cytopathic effect (c.p.e.) units ml^{-1}, was determined on porcine macrophage cultures as described by Martins et al. (1988).

Monocyte/macrophage culture

Cultures of porcine macrophages were prepared from heparinized blood essentially as described by Martins et al. (1988). Briefly, the leukocyte-rich fraction, prepared by Dextran flotation of blood, was centrifuged on a Ficoll–Hypaque gradient and the interface layer, containing peripheral blood mononuclear cells (PBMC), collected, washed and cultured in complete medium (RPMI-1640, 2 mM glutamine, 25 mM Hepes, 0.025% sodium bicarbonate, 10% autologous plasma and 10% fetal bovine serum (Hyclone Lab, UT)) that had been prescreened for low/no endotoxin. Macrophages grown with M-CSF were prepared essentially as described by Genovesi et al. (1989) in complete medium with 20% L929 cultured fibroblast supernatant. PBMC at 2×10^6 cells ml^{-1} were cultured in 150 cm^2 flasks and incubated at 37°C in 5% CO_2 atmosphere. For long term cultures, cells were washed in phosphate-buffered saline (PBS) and cultured in fresh complete medium without autologous plasma. Macrophages were purified by rinsing off adherent cells with cold calcium magnesium free PBS and then eluting adherent cells with PBS containing 0.03% EDTA for 20 min on ice. Eluted macrophages were pooled,

washed with Hanks balanced salt solution, and resuspended in complete medium.

ASFV infection of cultured macrophages

Three day old porcine macrophage cultures were harvested, and aliquots were cultured either without viruses or were adsorbed, in cell suspension, with 10 HAD_{50} units or 10 c.p.e. units of ASFV per cell for 1 h at 37°C. After three washes with RPMI-1640, the cells were incubated in complete medium with 2% fetal calf serum for the times indicated in the appropriate figures. Aliquots from each infected culture and from control cells (without virus) were taken at different time points, washed and used for antibody staining experiments. Control macrophage were also cultured with 0–2.5 ng ml^{-1} lipopolysaccharide (LPS) to assess endotoxin effects. Viability before and after infection was higher than 70% for all cultures as determined by Trypan blue exclusion.

Immunoblotting

The monoclonal antibodies (mAbs) used in these studies are listed in Table 1, along with their known reactivity against swine PBMC or ASFV antigens. Aliquots of $2-4 \times 10^6$ macrophages in 500 μl of RPMI-1640 were frozen and thawed three times using liquid nitrogen and tap water and microfuged for 20 min. The resultant supernatants were analyzed for protein content by Lowry assay (Lowry et al., 1951) and aliquots of these supernatants were frozen at −70°C until use. Duplicate samples (10 μg per sample) of each extract were applied onto presoaked nitrocellulose membranes (Schleicher and

TABLE 1

Reactivity of monoclonal antibodies

mAb	Specificity	Reference
74-22-15	Macrophage, granulocyte	Pescovitz et al., 1984
MSA4	CD2, pan T	Hammerberg and Schurig, 1986
76-7-4	CD1, B-cell subset	Pescovitz et al., 1984
PT85a	SLA Class I, monomorphic	Davis et al., 1987
74-11-10	SLA Class I, polymorphic	Pescovitz et al., 1984
TH16a	SLA-DQ, monomorphic	Davis et al., 1987
MSA3	SLA-DR, monomorphic	Hammerberg and Schurig, 1986
40D	SLA-DR, monomorphic	Lunney et al.,1983
135D4	VP73 ASFV	Whyard et al., 1985
82F8	VP12 ASFV	Whyard et al., 1985
174G11	VP32 ASFV	Whyard et al., 1985

Schuell A83, 2 mM). Nonspecific binding was blocked by 3 h incubation at room temperature with washing buffer (PBS containing 5% non-fat milk, 0.05% Tween 20, 0.01% sodium azide). Individual nitrocellulose strips were incubated overnight at 4°C with each of the mAb ascites, at 1:200–1:500 dilution in washing buffer. The strips of nitrocellulose paper were incubated with ^{125}I rabbit anti-mouse Ig, kindly provided by T. Whyard, PIADC, NY. The strips were washed, air dried and either autoradiographed directly, or the spots from nitrocellulose strips cut and radioactivity counted in a Beckman gamma counter.

Immunofluorescence

Cells were stained by indirect immunofluorescence and analyzed with a flow cytometer (FACSCAN, Becton-Dickinson, Sunnyvale, CA). Briefly, cells were washed twice with PBS and non-specific binding was blocked by incubating for 30 min at 4°C with PBS containing 5% normal rabbit serum. The cells were incubated with aliquots of the mAb supernatants listed in Table 1, for 40 min at 4°C, washed with PBS and incubated for another 40 min with fluoresceinated rabbit $F(ab')_2$-anti-mouse Ig. After washing, fluorescence data was collected on 10 000 light scatter gated macrophages using a FACSCAN Consort 30 program. Percentage of positive cells was calculated after setting the binding in the control samples, incubated with medium or mAb MSA4, to 5%.

The percentage of ASFV-infected cells was determined on aliquots of cells infected with each ASFV isolate. Cells were spun onto glass slides (cytospun) and fixed with methanol/acetone (50:50 v/v) at −20°C for 10 min and then air dried. The cells were preincubated with 1:5 dilution of normal swine serum for 20 min at room temperature, washed with PBS and incubated with fluoresceinated swine IgG against ASFV. The percentage of ASFV-infected cells was determined using a fluorescent microscope.

Dual antibody staining of macrophages

The procedure for dual antibody labeling has been described in detail by Gonzalez Juarrero et al. (1991) and was adapted for lightly fixed cytospun cells. Briefly, $2–4 \times 10^4$ macrophages in 200 μl of RPMI-1640 were cytospun and fixed with 1% paraformaldehyde, 0.1 M lysine, 0.01 M sodium *m*-periodate (PLP buffer) (pH 7.4) for 10 min at room temperature. Non-specific binding was blocked by 1% normal pig serum in PBS for 30 min prior to 1 h incubation with mAb culture supernatant or medium as a control. Between all steps TBS (0.85% NaCl, 0.1 M Tris, pH=7.8) washes were performed. Alkaline phosphatase coupled rabbit anti-mouse Ig conjugate (Dako Co., Copenhagen, Denmark) was reacted with cells. Finally substrate, naphthol AS-

MX, was added and positive staining was visualized in blue. The second staining was performed by incubating cells with biotinylated porcine IgG anti-ASFV prior to blocking endogenous peroxidase with 0.1% phenylhydrazine. Peroxidase coupled avidin biotin complex (Vector Labs, Burlingame, CA) was added followed by the substrate, 3-amino-9-ethylcarbamizole, in the presence of H_2O_2. Positive reaction for this staining was red. Slides were then mounted in glycerol gelatin GG (Sigma) and examined under a light microscope.

RESULTS

Expression of SLA antigens on cultured porcine macrophages

Macrophages at different intervals of culture were tested for total antigen expression by immunoblotting assays and for cell surface antigen expression of macrophage-specific markers and SLA by immunostaining and flow cytometric analyses. The time course assessment of total antigen expression in macrophage cultures, examined by immunoblotting assays, showed that macrophages cultured without M-CSF exhibited maximal expression of SLA Class I (mAb PT85a, 74-11-10) and macrophage markers (mAb 74-22-15) by Day 3 of culture (Fig. 1(A)). Similar results were found for SLA Class II; in addition, at Day 4 there was a greater decrease in SLA-DR (mAb MSA3 and 40D) than SLA-DQ (mAb TH16) antigen expression. In a series of experiments, the day of maximum expression for these antigens varied but the peak of reactivity was consistently found at Days 3 and 4 of culture (data not shown). A major decrease of SLA Class I, Class II and macrophage markers was observed by Day 6 of culture without M-CSF (Fig. 1(A)). The same macrophages cultured in the presence of M-CSF (Fig. 1(B)) also exhibited maximal expression of SLA Class I and Class II at Day 3 although the macrophage marker (mAb 74-22-15) exhibited no differential expression during the time of culture. At Days 6 and 15 of culture, and in contrast with macrophages cultured without M-CSF, SLA Class I expression was still pronounced whereas expression of SLA Class II was minimal when compared to control (mAb MSA4) values (Fig. 1(B)).

Quantitative analyses of cell surface expression of SLA and macrophage specific markers in both types of cultures was obtained using immunostaining and flow cytometric analyses, reporting two parameters: the percentage of positive cells and the intensity of fluorescence or mean channel fluorescence. The total number of SLA Class I positive cells was not changed but rather the number of antigens per cell, as indicated by the mean channel intensity of fluorescence of the cell population, was increased with M-CSF, a change of 300 channels or more (Fig. 2(B)) and without M-CSF, a change of 150 channels or more (Fig. 2(D)). These increases in marker expression peaked at 3–

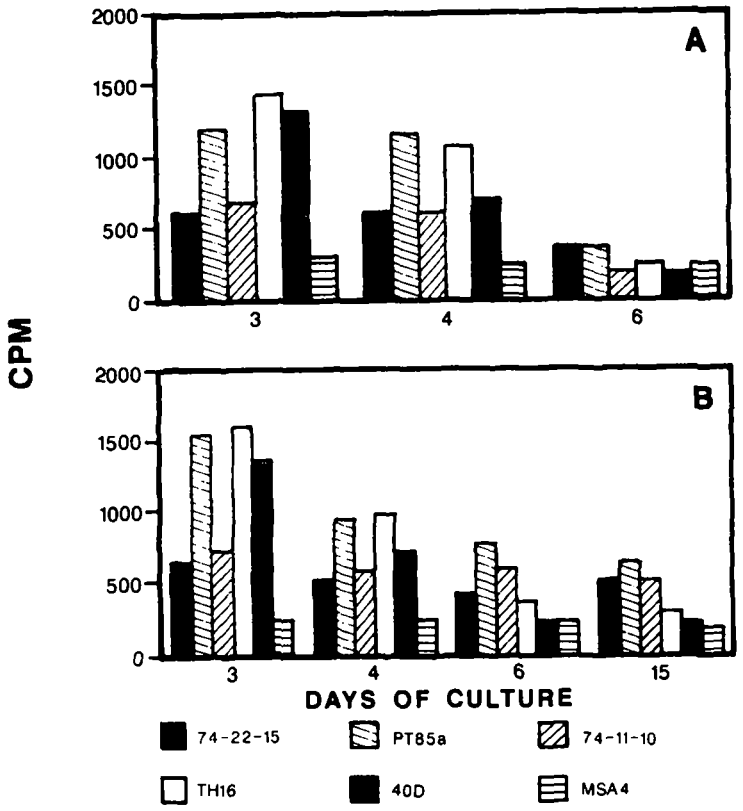

Fig. 1. Effect of M-CSF on antigen expression of porcine macrophage cultures. Porcine macrophages were cultured without (A) or with 20% L929 conditioned medium (M-CSF) (B). The immunoblotting assay was performed on freeze–thaw extracts of porcine macrophage cultures harvested on the days noted. Ten micrograms of freeze–thaw extracts of porcine macrophages were spotted onto nitrocellulose paper and incubated with mAb against T-cells (MSA4) as a background control, macrophage markers (74-22-15), SLA Class I (PT85a, 74-11-10) and SLA Class II (MSA3, 40D, TH16a) followed by incubation with ^{125}I-coupled rabbit IgG anti-mouse Ig. Washed strips were air dried, cut and each spot counted in the gamma counter. Average values of duplicate samples are shown. The control, represented by the CD2 T-cell mAb MSA4, was equal to or greater than the control with medium run simultaneously.

4 days of culture and thereafter decreased, in agreement with the results shown by immunoblotting (Fig. 1).

Changes in the expression of SLA Class II on macrophages during culture were reflected both in the percentage of cells that express Class II antigens as well as in the intensity of marker expression. Macrophages cultured without M-CSF increased from 40% (TH16, SLA-DQ) or 65% (40D, SLA-DR) Class II positive at 1 day of culture to 60% and 80% positive, respectively, at 3 or 4 days of culture with a mean fluorescence increase of approximately 100 channels for SLA-DR and 30 channels for SLA-DQ (Fig. 2A,2B). These increases

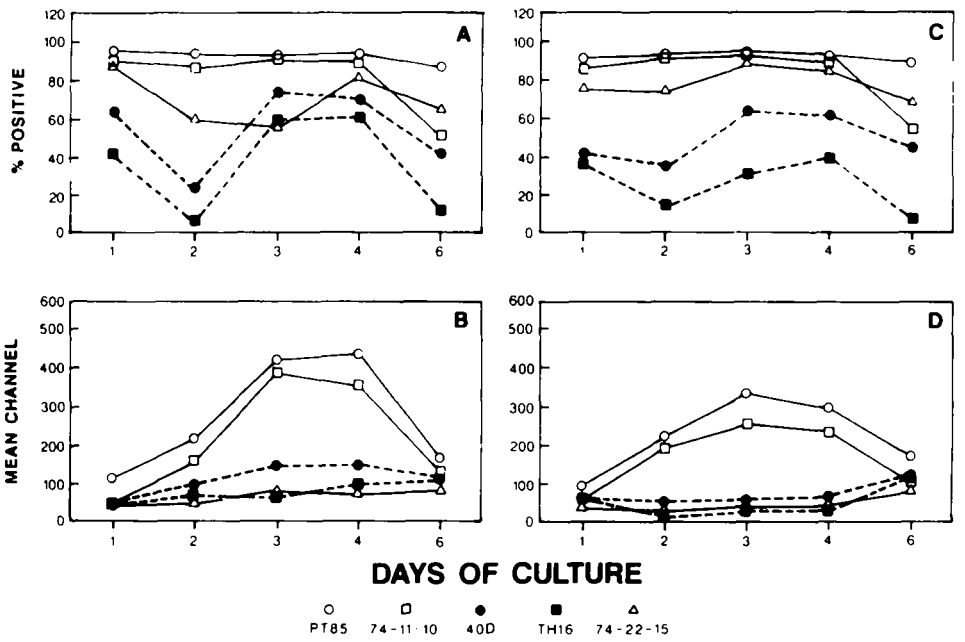

Fig. 2. Surface antigen expression on cultured porcine macrophages without or with M-CSF. Percentage of positive cells (A,C) and fluorescence mean channel (B,D) of porcine macrophages cultured at 1, 2, 3, 4, and 6 days without M-CSF (A,B) and with M-CSF (C,D), and stained for macrophage marker (mAb 74-22-15), SLA Class I (mAb PT85a, 74-11-10), and SLA Class II (mAb TH16, 40D). All samples were analyzed in a FACSCAN (Becton Dickinson) using the same side scatter and forward scatter gates and the same detector level for FL1. The integration for each set of samples were adjusted to 5% positive cells in the control sample without specific antibody.

were reversed by 6 days of culture. When macrophages were cultured with M-CSF they exhibited a less pronounced increase in the percentage of SLA II positive cells, although these still peaked at 3–4 days of culture (Fig. 2(C)). The relative intensity of SLA Class II expression was changed little in macrophages cultured with M-CSF, although the remaining Class II positive cells may be slightly brighter for SLA Class II expression at 6 days of culture (Fig. 2(D)). Incubation with LPS endotoxin caused no such increase in SLA I or II expression (data not shown). Overall, the changes in SLA Class II expression varied significantly between experiments. Thus, macrophages harvested after 1 day of culture ranged from 30 to 75% positive for SLA Class II expression, however, the maximum percentage of Class II positive cells invariably occurred at 3–4 days of culture with or without M-CSF.

Effect of ASFV infection on macrophage antigen expression

Based on our initial observations of SLA Class I and Class II expression on normal cultured macrophages, we analyzed whether ASFV replication would

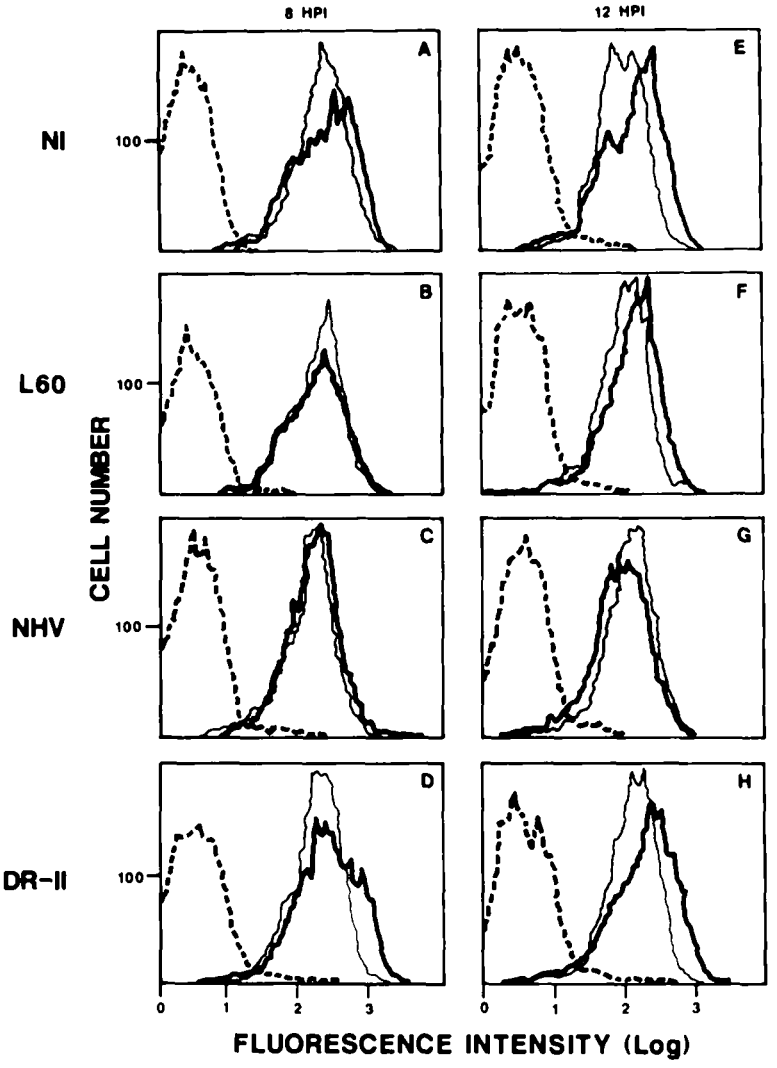

Fig. 3. Effect of ASFV on porcine macrophage surface antigen expression. Histograms were obtained on immunostained macrophages using the Consort 30 programs and FACSCAN analyses (Becton Dickinson), and represent the number of cells versus log fluorescence intensity. Porcine macrophages were not-infected (NI) (A,E) or were infected at the times noted with the highly virulent L60 (B,F), with the non-virulent NHV (C,G), or with the moderately virulent DR-II (D,H) ASFV isolates. Aliquots of cells were washed, incubated with mAb MSA4 (---), as a control, 74-22-15 (——) macrophage marker, or PT85a (——) SLA Class I, followed by fluoresceinated rabbit $F(ab')_2$ anti-mouse Ig. All samples were analyzed on FACSCAN as described in Materials and Methods. The percentage of positive cells for PT85a or 74-22-15 was always higher than 95%, using the same integration points set for MSA4 as the control mAb.

TABLE 2

Effect of ASFV infection on SLA and macrophage antigen expression

Time (hpi)	ASFV isolate	74-22-15	PT85a		TH16a		40D	
		Mean	Mean	(ratio)	Mean	(ratio)	Mean	(ratio)
8	None	321	375	(1.17)	37	(0.11)	35	(0.11)
	L60	289	273	(0.94)	28	(0.10)	36	(0.12)
	NHV	208	217	(1.04)	21	(0.13)	28	(0.13)
	DR-II	259	429	(1.66)	31	(0.12)	45	(0.17)
12	None	145	247	(1.70)	41	(0.32)	89	(0.61)
	L60	169	232	(1.37)	66	(0.39)	56	(0.33)
	NHV	186	149	(0.80)	40	(0.21)	74	(0.40)
	DR-II	185	305	(1.65)	66	(0.36)	72	(0.39)

Three-day cultured macrophages were harvested and aliquots were infected with ASFV. At 8 or 12 h post-infection (hpi) cells were harvested and stained for macrophage antigen (74-22-15), for SLA Class I (PT85a) or SLA Class II (TH16a, SLA-DQ, or 40D, SLA DR) expression. Separate analyses showed that more than 95% of cells were infected with virus. FACSCAN analyses revealed that 74-22-15 and PT85a stained more than 96% of the cells whereas TH16a and 40D stained 32–79% of the macrophages (Figs. 3 and 4). Similar results were found in another complete repeat of this experiment as well as in several smaller trial experiments. Intensity of antigen expression is designated by the mean channel of the fluorescent signal; the ratio is the mean channel of the PT85a, TH16a or 40D fluorescence divided by the mean channel of 74-22-15 fluorescence of aliquots of the same macrophage preparation.

affect SLA expression. Assays were performed at times when ASFV replication was evident in the cytoplasm and prior to virus shedding and cell death. Using high multiplicity of infection (10 HAD_{50} or 10 c.p.e. per cell), more than 90% of macrophages were infected with the virus and virus replication was evident by 4 h, whereas by 24 h major cell losses were evident. Total antigen expression was assessed by immunoblotting, and surface antigen expression by immunostaining and flow cytometric analyses. Since preliminary experiments indicated that the same pattern of antigen changes after ASFV infection occurred with 2-, 3- or 4-day cultured macrophages, all experiments reported below were performed with 3-day cultured macrophages. Even though cultured macrophages exhibited more variable antigen expression after culture than cell lines, such as Vero cells, macrophages were chosen for these studies because of their relevance as the true target of ASFV infections.

Macrophage antigen expression was assessed using mAb 74-22-15 which reacts weakly with the immunoblotted antigen. When extracts from non-infected macrophages were compared with extracts from macrophages infected for 4, 8, 12, or 24 h with L60, DR-II or NHV isolates there was no apparent difference in 74-22-15 antigen expression (data not shown). When surface

antigen expression was assessed by immunostaining of intact macrophages at 8 and 12 h after ASFV infection, there was no decrease in the percentage of 74-22-15 positive cells (Fig. 3), however, there was a decrease in the intensity of antigen expression at 8 h after infection with all of the isolates, which was most pronounced with the NHV isolate (Table 2). At 12 h post-infection, however, the expression of the 74-22-15 antigen was increased in the ASFV-infected cells when compared to the non-infected cells harvested at the same time point, although the surface 74-22-15 expression of all aliquots appeared to be decreased.

SLA Class I expression following ASFV infection

Immunoblot analyses of total SLA Class I expression in extracts of macrophages infected with ASFV for 4, 8, 12, or 24 h indicated that there were no major differences (data not shown). When surface expression of intact macrophages was analyzed a different result was obtained; all macrophages expressed the SLA Class I marker but at a much lower intensity for L60 and NHV-infected macrophages. At 8 and 12 h post-infection, ASFV infected macrophages had less SLA Class I on the surface than non-infected macrophages (Figs. 3, 4(A,B)). Since the infected cells expressed less macrophage antigen at 8 h post-infection there could have been a general decrease in expression of surface antigens: to assess this, the relative intensity of the two markers, i.e. macrophage antigen and SLA, were compared as a ratio of the mean channel of fluorescence intensity of SLA relative to 74-22-15. The ratio calculations indicated that SLA Class I expression was reduced in macrophages infected for 8 h with the L60 or NHV ASFV isolates relative to the same macrophages cultured in medium alone (Table 2). Similar ratios were found at 12 h post-infection. For the moderately virulent DR-II ASFV isolate, however, SLA Class I expression was increased at both time points using either measure of SLA Class I intensity.

Class II expression following ASFV infection

The wide degree of variation of SLA Class II expression on cultured macrophages makes studies of the effect of viral infection on SLA Class II expression more difficult. The immunoblotting profiles showed that total Class II expression is decreased after 8 h of culture in both infected and non-infected macrophages (data not shown). Comparison with ASFV-infected cells revealed apparently higher levels of SLA Class II expression at 8 h post-infection but no apparent differences at 12 or 24 h. Assessment of surface antigen expression indicated that the intensity of SLA-DQ expression decreased at 8 and 12 h after infection (Fig. 4(C,D)). Similar results were found for SLA-

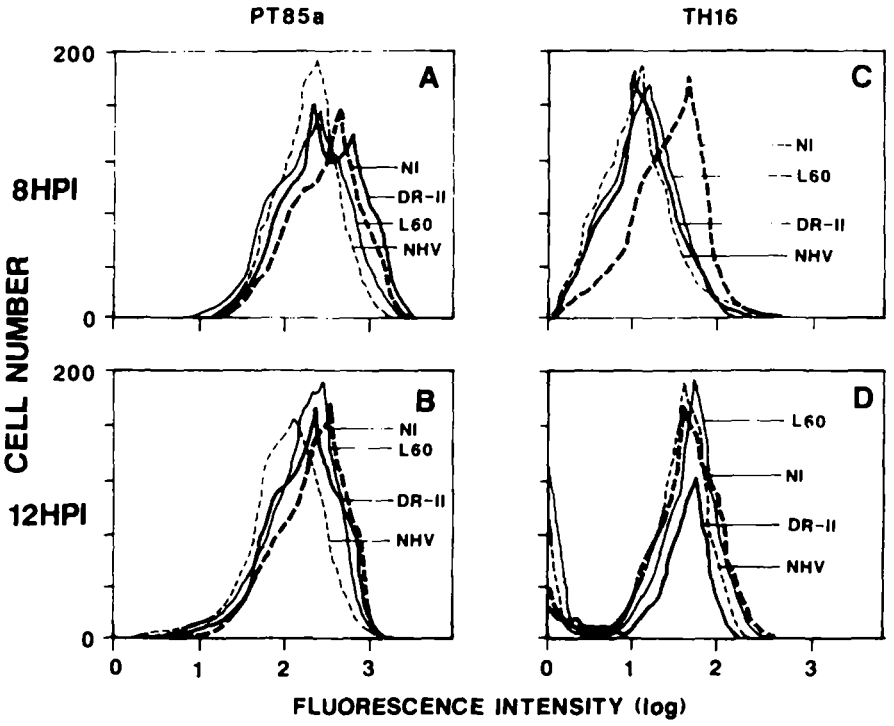

Fig. 4. Effect of ASFV on porcine macrophage surface SLA expression. Overlapping histograms were obtained with the Consort 30 program of FACSCAN (Becton Dickinson) and represent the number of cells versus $\log_{10}$ of fluorescence intensity. Porcine macrophages were not-infected (NI) or infected for 8 (A,C) and 12 (B,D) hours post-infection (HPI) with the highly virulent L60, with the non-virulent NHV, or with the moderately virulent DR-II ASFV isolates. Aliquots were incubated with mAb PT85a, SLA Class I (A,B), or TH16, SLA-DQ Class II (C,D), followed by incubation with fluoresceinated rabbit $F(ab')_2$-anti-mouse Ig. All samples were analyzed in a FACSCAN as described in Materials and Methods. Similar results were found in another complete repeat experiment as well as in several smaller trial experiments.

DR expression based on both mean channel and ratio determinations (Table 2).

Viral antigen expression

Viral antigen expression was demonstrated during the course of infection with ASFV. Porcine macrophages expressing viral proteins intracellularly were detected as early as 4 h post-infection using either the immunoblot assay or staining of permeabilized cells with porcine IgG against-ASFV (Fig. 5). Macrophage surface expression of ASF viral antigens could not be determined at early times post-infection using different anti-ASFV mAb (Table 1) or polyclonal porcine IgG against ASF viral proteins. Using fluorescence immuno-

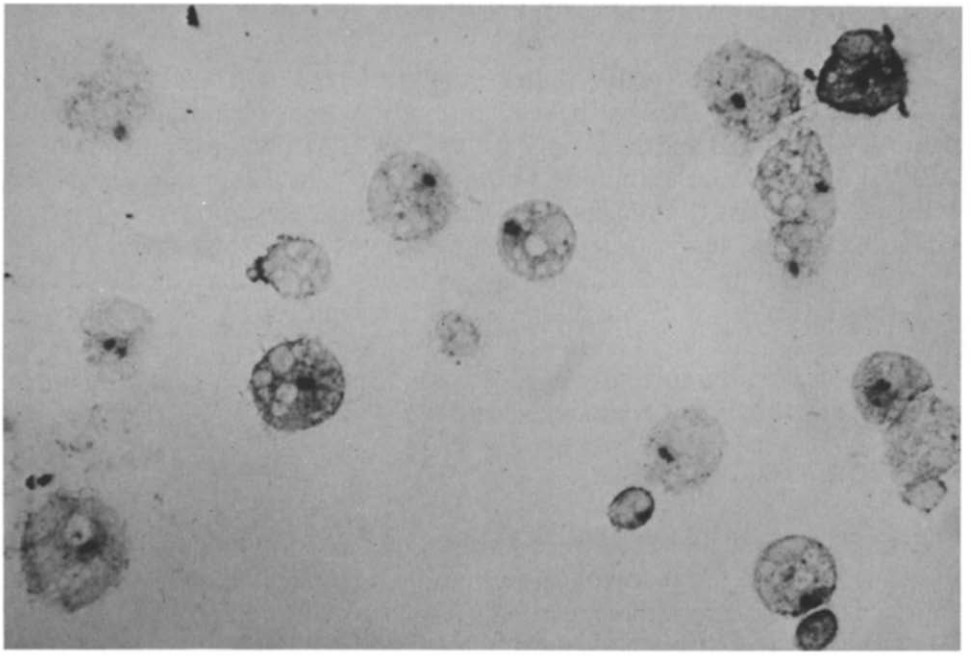

Fig. 5. Dual expression of SLA Class II and ASFV antigens. Dual antibody staining of porcine macrophages infected with ASFV L60 isolate for 12 h. Cytospun macrophages were stained with anti-SLA Class II mAb, MSA3, and anti-mouse IgG coupled to alkaline phosphatase (blue) and then with biotinylated porcine IgG against ASFV antigen coupled to biotin and avidin peroxidase (red). Original magnification ×200.

staining, there was positive surface staining for ASFV antigens between 12 and 24 h post-infection but on only 25–30% of infected cells as compared to 90% of the same macrophages expressing intracellular viral proteins observed by staining cells after permeabilization (Fig. 5). This discrepancy could be explained by the lack of mAb, and swine antisera, reactive with ASFV epitopes expressed on the infected macrophage surface.

Anti-SLA mAb do not block ASFV infection of macrophages

The ability of mAb against SLA to block ASFV infection was studied by incubating porcine macrophages, prior to infection with ASFV L60, with mAb against SLA Class I (PT85a), Class II (MSA3), or T-cells (MSA4). At 12 h post-infection cells were stained with fluoresceinated swine IgG against ASFV and the number of infected cells was counted for each sample. No differences in the percentage of infected cells was found when macrophages were treated prior to infection with mAb against SLA Class I, Class II, or without mAb (data not shown).

Dual expression of SLA Class II and ASF antigens

The possibility that ASFV preferentially infected SLA Class II positive macrophages was studied by the dual antibody staining technique using mAb against SLA Class II and swine IgG against ASFV. At 12 h post-infection with ASFV L60 two color immunostaining showed that both macrophages expressing SLA Class II antigens (blue) and macrophages not expressing SLA Class II antigens were infected by ASFV (red) (Fig. 5). Moreover, the percentage of ASFV infected cells was always higher (90%) than the percentage (30–70%) of cells expressing SLA Class II before infection. When cells were analyzed at 4, 8, 12, or 24 h after infection with the L60, DR-II or NHV isolates, no preferential infection of SLA Class II positive or negative cells was found at any time point (data not shown).

DISCUSSION

Our results indicate that expression of SLA Class I and Class II is greatly enhanced on porcine macrophages in cultures independent of M-CSF. The up-regulation, which is maximal at 3 to 4 days of culture for SLA Class I and Class II, is found for the total, immunoblotted SLA as well as for the antigens expressed on the cell surface. Fluorescence analyses verified that there is a major upward shift in the number of SLA Class I molecules on each macrophage whereas for SLA Class II there is both a novel expression of SLA Class II and an upward shift in the number of molecules per cell, which is greatest on macrophages cultured without M-CSF. By 6 days of culture these increases are reversed. These results indicate that 3-day-old macrophage cultures should be suitable stimulators of immune responses and the best targets for SLA-mediated cytolytic activities.

These changes in expression of SLA Class I and Class II markers correlate with the growth, survival and functional data of such cultured swine macrophages (Martins et al., 1988; Genovesi et al., 1989). While maximal cell proliferation occurs between 4 and 6 days of culture with M-CSF, the peak of SLA expression was restricted to 3 to 4 days of culture. In cultures with M-CSF, cell proliferation declined by 6–8 days of culture and the expression of SLA Class I and Class II declined at 6 and 15 days of culture, respectively. The expression of MHC Class II antigens on monocytes and tissue macrophages from other animal species such as murine and human, also decreased after prolonged culture (Gonwa and Stobo, 1984; Toews et al., 1984).

After infection with NHV and L60 ASFV isolates macrophages exhibited decreased Class I antigen expression at 8 or 12 h post-infection; in contrast, an increase of SLA Class I was seen on cells infected with ASFV DR-II. Interestingly, these changes in SLA Class I expression do not seem to correlate with the virulence of ASFV isolates (Pan and Hess, 1984). The general losses in

74-22-15, SLA Class I and Class II could be attributed to virus mediated membrane turnover as virus infects the cells and moves to the replication centers in the cytoplasm. However, this would not explain the increase in SLA Class I expression on DR-II-infected macrophages. This isolate may express a unique function, possibly associated with other moderately virulent ASFV isolates, in that it causes up-regulation of SLA Class I expression similar to that observed after infection with radiation–leukemia virus, adenovirus 12, or coronavirus (Brown et al., 1988). The fact that the non-virulent NHV isolate does not also stimulate SLA I expression may be due to the NHV isolate being related to the virulent L60 isolate or to it requiring other cells, e.g. T-cells, to secrete factors to influence macrophage antigen expression. The apparent recovery of some SLA antigen expression at 12 h post-infection may be due to a concomitant decrease in viral protein synthesis which in Vero cells is maximal at 8 h post-infection (Tabares et al., 1980).

The decrease in SLA Class I surface expression found in macrophages infected with ASFV NHV or L60 was not detectable when total extracts of these macrophages were analyzed by immunoblot analyses. This may indicate that the relative loss of surface SLA is small compared to the total cell associated SLA and therefore not detectable by the assay system. It is more likely that the deficit in surface SLA expression is due to a reduction in the cycling of the antigens back to the cell surface. This is supported in part by an electron microscopic examination of macrophages which reveals that SLA Class I and Class II molecules are associated with internal ASF viral particles detected by mAb and immunogold staining (M. Gonzalez Juarrero et al., unpublished data, 1988). As these viral particles bud from the macrophage surface at later times in the infection, they may carry these SLA proteins in the viral envelope, similar to the earlier report of ASFV budding from Vero cells (Carrascosa et al., 1985). A decrease in expression of SLA Class II may lead to a decrease in antigen presentation, a mechanism favorable for survival of the virus.

Some viruses, such as lactic dehydrogenase virus (Inada and Mims, 1985), Semliki Forest virus (Helenius et al., 1978) and cytomegalovirus (Grundy et al., 1987) are reported to use MHC antigens as viral receptors. In vitro studies of pig monocytes infected with ASFV showed that both Class II positive and negative pig monocytes were equally likely to be infected with ASFV. Moreover, treatment of these monocytes before infection with mAb against SLA Class I or Class II caused no decrease in the number of ASFV-infected cells. These results indicated that there is no preferential infection of SLA Class II positive cells by ASFV and that at least the epitopes recognized by the SLA Class II mAb are not involved as receptors for ASFV.

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