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Antibodies to human and non-human primate cellular and culture medium components in macaques vaccinated with the simian immunodeficiency virus

L. A. BERGMEIER, J. WALKER, L. TAO, M. CRANAGE* & T. LEHNER *Division of Immunology, United Medical and Dental School of Guy's and St Thomas' Hospitals, London and *Pathology Division, PHLS CAMR, Salisbury*

SUMMARY

Inactivated simian immunodeficiency virus (SIV) grown in a human T-cell line induces protection from infection by the virus in macaques. However, observations that immunization with uninfected human T cells or with SIV-1 prepared in human T cells can also induce protection, has raised the possibility that protective antigens could be of human cellular origin. Sera from animals immunized with fixed infected and uninfected human T cells, as well as from animals immunized with partially purified cell-free SIV have been examined for their ability to bind to human and macaque peripheral blood mononuclear cells (PBMC) and to components present in fetal calf serum (FCS) in which the cells were grown. Analysis by flow cytometry suggests that antibodies to human cell surface antigens can be elicited with both inactivated SIV grown in human T cells and by uninfected T cells. There was a significant association between the presence of anti-cell antibodies and protection from infection. However, anti-cell surface antibodies were not detected with macaque mononuclear cells by flow cytometry or by immunoprecipitation, unless these cells were first treated with FCS or activated by a mitogen. Immunoprecipitation of resting human PBMC with sera from immunized animals suggests the presence of antibodies to class I heavy and light chains [β_2-microglobulin (β_2m)] and to bovine β_2m, which may originate in FCS used to grow the cell line. Antibodies to CD4 were also found in sera from animals immunized with SIV grown in human T cells. We suggest that human cellular components augmented by FCS elicit anti-class I heavy chain, β_2m, CD4 and FCS antibodies which may be responsible for protection against SIV infection in macaques.

INTRODUCTION

Inactivated simian immunodeficiency virus (SIV) grown in human T cells induces protection against SIV infection and simian acquired immune deficiency syndrome (AIDS).[1–8] However, cellular material in which viruses are grown have been known for some time to influence the immunogenicity of the vaccine.[9] Retroviruses can incorporate cellular antigens as they bud from infected cells.[10] Indeed, HLA class I heavy and light chains [β_2-microglobulin (β_2m)], and class II antigens were found in human immunodeficiency virus (HIV) grown in the human H-9 T-cell line.[10,11] Cellular material in which SIV was grown received a great deal of attention with the report that immunization with uninfected human T cells in which SIV was grown, also induced protection against SIV infection.[12] Investigations in the UK and USA suggest that antibodies against human T-cell surface antigen showed significant correlation with protection against SIV infection[12,13] and these antibodies react with major histocompatibility complex (MHC) class I antigens.[14–16] Indeed, immunization with SIV grown in human T cells, but challenged with live SIV grown in simian cells, failed to prevent SIV infection in most macaques.[17–19] HLA class I antigens, both the heavy and β_2m chains, are found in viruses[9] and in HIV grown in T-cell lines.[10] HLA class II antigen has also been reported in HIV grown in human T-cell lines,[11,15] suggesting that immunization with these viruses may elicit allogeneic responses to HLA class I and class II antigens.

The components of the culture medium in which the cells are grown may also be immunogenic. This applies particularly to β_2m in fetal calf serum (FCS) which readily exchanges with human β_2m in HLA class I antigen,[20] making the latter more foreign to the macaque and thereby probably increasing its immunogenicity. In addition to HLA antigens and components of FCS, the CD4 glycoprotein expressed by T cells in which the SIV is grown is also immunogenic. This is potentially another important mechanism, as antibodies to CD4 have been shown to suppress the ability to recover virus from SIV-infected macaques and could therefore have therapeutic value.[21]

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Abbreviations: β_2m, β_2-microglobulin; Con A, concanavalin A; FCS, fetal calf serum; PBMC, peripheral blood mononuclear cells; SIV, simian immunodeficiency virus.

Correspondence: Ms L. A. Bergmeier, Department of Immunology, United Medical and Dental Schools, Guy's Hospital, London SE1 9RT, UK.

The objectives of this investigation were to identify cellular and humoral responses to four potential immunogenic components: T-cell surface antigens, especially HLA classes I and II, CD4 and antigens found in FCS. We were also concerned that several studies[13–16] demonstrated anti-cell antibodies only to human cell surface antigens, although anti-macaque antibodies were detectable in whole-cell lysates from macaque peripheral blood mononuclear cells (PMBC) (M. Cranage, unpublished observations). We report here antibodies to human $CD3^+$, $CD4^+$, $CD8^+$ T cells, B cells and macrophages. Anti-simian cell antibodies were also detected, provided the simian cells were treated with FCS or activated by a mitogen. Significant cell surface, β_2m and FCS antibodies were found in sera from SIV-protected compared with SIV-infected macaques. The antibodies recognized HLA class I heavy chains and β_2m (both human and bovine) and CD4 glycoprotein. We suggest that protection against SIV infection in macaques is induced by human cell surface antigens, augmented by FCS components, which elicit anti-HLA class I heavy chain, β_2m, CD4 and FCS antibodies.

MATERIALS AND METHODS

Macaque sera

A series of 39 sera was used from immunized and control macaques. Sera were examined from 12 rhesus and four cynomolgus macaques immunized i.m. (four to five times) with cell-free partially purified inactivated SIVmac-32H (500 or 100 μg/dose), grown in human C8166 cells; the adjuvant was either aluminium hydroxide or threonyl muramyl peptide in the SAF-1 vehicle (Syntex Research, California, CA). Another series of sera was analysed from 10 cynomolgus monkeys, immunized i.m. (five times) with fixed uninfected C8166 cells in Quill A adjuvant. All macaques were challenged 2 weeks after the final immunization i.v. with live SIVmac-32H grown in human C8166 cells. Unimmunized control sera were analysed from 13 macaques (seven rhesus and six cynomolgus), four of which were immunized with fixed uninfected rabbit RK13 cells; five preimmunization sera were also used.

Flow cytometry of human and macaque cells

Human mononuclear cells were separated by standard Hypaque–Ficoll density gradient centrifugation and treated with serum from a macaque immunized with partially purified SIVmac vaccine or from an unimmunized control. This was followed by affinity-purified goat anti-monkey IgG antibodies and then by rabbit anti-goat fluorescein isothiocyanate (FITC) (Nordic Laboratories, Maidenhead, UK). The subsets of cells reacting with these antibodies were determined by double fluorescence flow cytometry, by further incubation with biotinylated CD3, CD4 or CD8 monoclonal antibodies (mAb) followed by streptavidin–phycoerythrin (Becton Dickinson, Mountain View, CA) or phycoerythrin-conjugated CD20 or CD14 antibodies.[22] The cells were analysed on the Epics Profile II flow cytometer (Coulter Electronics, Haileah, FL) and 5000 cells were analysed to generate each histogram. The percentage of cells and mean fluorescence intensity were recorded. Similarly, macaque mononuclear cells were separated and either incubated in RPMI containing 10% FCS for 16 hr or activated with concanavalin A (Con A; 25 μg/ml) for 2 days. The resting, FCS and Con A-activated cells were then treated with sera from SIVmac-immunized and control macaques, and then analysed by flow cytometry. Cells from individual donors were used for each experiment which was carried out on more than three macaques.

Anti-human cell antibodies and enzyme-linked immunosorbent assay (ELISA)

Anti-human cell antibodies were determined by flow cytometry (as described above). The results are expressed as the per cent of reacting cells, with the anti-goat FITC values subtracted. ELISA was used[23] to determine antibody titres to FCS (Gibco; Life Sciences, Paisley, UK), human β_2m (Serotec, Kidlington, UK), and BSA (Sigma Chemical Co, Poole, UK). Briefly, plates coated with antigen (at 1 μg/ml) were incubated with doubling dilutions of the test serum. Bound antibody was detected by incubating with rabbit IgG anti-monkey IgG (2 μg/ml; Nordic Laboratories), followed by affinity-purified goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma Fine Chemicals, Poole, UK) and *p*-nitrophenyl-phosphate-disodium (Sigma Diagnostics, Poole, UK). The reaction was terminated with 3 M NaOH and the optical density was measured at a wavelength of 405 nm. The results were expressed as the highest dilution giving an OD > 0·2 units above the background sample. Anti-bovine serum albumin (BSA) antibodies were not detected in any sera.

Absorption studies

Increasing concentrations of FCS, human β_2m, bovine β_2m (a gift from Dr M. L. Groves, Philadelphia, PA), and BSA were coupled to cyanogen bromide-activated Sepharose 4B beads (Kabi-Pharmacia, Milton Keynes, UK); 2 mg of the antigen was mixed with 0·5 g of the activated beads, in 0·1 M bicarbonate coupling buffer, for 2 hr at room temperature. Unreacted end groups were blocked by incubation in 1 M ethanolamine (pH 8·0) for 2 hr at room temperature. The antigen-coated beads were washed in three cycles of 0·1 M acetate buffer, containing 0·5 M NaCl (pH 4·0), followed by 0·1 M borate buffer, containing 0·5 M NaCl (pH 8·0). The beads were resuspended in phosphate-buffered saline (PBS) and the macaque sera (at dilutions of 1:100, 1:200, etc.) were then mixed with the beads at 37° for 1 hr and overnight at 4°. The beads were pelleted by centrifugation and the supernatants were assayed against uninfected and SIV-infected C8166 cells by ELISA.

Lactoperoxidase cell surface labelling and immunoprecipitation of human cells[24]

Human PBMC were separated on Hypaque–Ficol, washed three times in PBS and resuspended at 10^8 cells/ml. Aliquots of 100 μl of the cell suspension were treated with 10 μl lactoperoxidase, 20 μl glucose oxidase (Sigma Fine Chemicals Ltd), 5 μl ^{125}I (Amersham International, Amersham, UK) and 10 μl glucose (Sigma Fine Chemicals Ltd). The iodination was allowed to proceed for 10 min at room temperature, the cells were then washed three times in PBS, resuspended in 1 ml of lysis buffer [10 mM Tris HCl pH 7·4, containing 1% Nonidet P-40 (NP-40), 1 mM EDTA, 0·1 M PMSF, 150 mM NaCl], supplemented with BSA at 1 mg/ml, and kept on ice for 30 min. The lysate was centrifuged for 5 min at 2000 *g* and the supernatant further centrifuged for 1 hr at 100 000 *g* to remove nuclear and subcellular debris. Immunoprecipitation was carried out using fixed *Staphylococcus aureus* (Pansorbin,

Calbiochem, La Jolla, CA). Lysates of 10^7 cells were precleared by incubation for 30 min at 4° with 100 μl of a 10% w/v suspension of bacteria in lysis buffer supplemented with 1 mg/ml of BSA. Bacteria were removed by centrifugation (12 000 *g* for 15 min at 4°) and the supernatants supplemented to 0·5 M NaCl before incubating with 10 μl of macaque sera (unimmunized or immunized with either uninfected cells, infected cells or partially purified virus), polyclonal or mAb (ascites) or with 50–100 μl of mAb (supernatants). If IgG1 or IgM mouse monoclonal cells were used, then either a second antibody, rabbit anti-mouse IgG or IgM was added to form a protein-A binding immune complex, or Sepharose beads conjugated with anti-mouse IgG1 or IgM were used. The immobilized immune complex was then pelleted by centrifugation at 4° and the pellets washed with lysis buffer, containing 0·5 M NaCl, followed by lysis buffer containing 0·1% sodium dodecyl sulphate (SDS), and finally 0·1 M Tris–HCl (pH 7·4) and 0·1% NP-40. The pellets were resuspended in 50 μl of SDS–PAGE (polyacrylamide gel electrophoresis) reducing buffer, boiled at 100° for 5 min and the solubilized proteins were separated on 10% SDS–PAGE gels. Electrophoresis was carried out at 200 volts for 45 min. The gels were stained with 0·1% Coomassie Blue R and dried under vacuum onto Whatman 3 mm paper and autoradiographed, using Kodak X-OMAT-S film with Ilford intensifying screens for 2–7 days.

Lactoperoxidase cell surface labelling and immunoprecipitation of macaque cells were carried out with untreated cells, cells incubated for 16 hr in RPMI with 10% FCS, and cells activated with Con A (25 μg/ml) for 2 days. The same technique was then used as described for human cells. The lysates were precipitated with the following antibodies: (1) control monkey serum; (2) SIV-vaccinated monkey serum; (3) rabbit anti-FCS antibodies; (4) rabbit anti-human β_2m antibodies; (5) anti-human HLA class I mAb (W632, Dako, High Wycombe, UK); (6) anti-human HLA class II mAb (Becton Dickinson, Cowley, UK); (7) anti-FCS followed by SIV vaccinate; and (8) anti-human β_2m followed by SIV vaccinate.

Western blots

Recombinant CD4 (MRC AIDS directed programme, ADP no. 608/609) was boiled for 5 min in SDS–PAGE reducing buffer containing 2-mercaptoethanol (Sigma Fine Chemicals) and 10 μg was applied to each track. SDS–PAGE was then carried out at 180 volts for 45 min, using 10% gels in a discontinuous buffer system.[25] The separated protein was transferred onto nitrocellulose sheets (Gelman Sciences, Ann Arbor MI), using the Bio-Rad transblot apparatus (Bio-Rad, Watford, UK), at 60 volts for 30 min. The nitrocellulose sheets were then cut into strips and blocked with skimmed milk protein in PBS for 16 hr at 4°. The strips were washed three times in PBS/Tween-20 (PBS/T20), the primary antibodies were then applied (diluted in 0·5% BSA in PBS/T20) and incubated overnight at 4°. This was followed by incubation with alkaline phosphatase-conjugated indicator antibody of the appropriate species; rabbit anti-monkey IgG, goat anti-rabbit IgG or rabbit anti-mouse conjugate (Sigma Fine Chemicals) at a dilution of 1:500. The enzyme conjugate was incubated as for the primary antibody and the reaction was developed with 100 mM Tris–HCl pH 9·0 buffer, containing 100 mM NaCl and 50 mM MgCl, nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphatase (BCIP) (Sigma). The enzyme reaction was stopped by washing the strips in 1 mM EDTA. CD4 was blotted with the following antibodies: Leu-3a antibodies, SIV-vaccinated monkey serum, control monkey serum, rabbit anti-FCS, rabbit anti-human β_2m and normal rabbit serum.

RESULTS

Flow cytometry of human cells

Examination of sera from rhesus and cynomolgus macaques immunized with SIV MAC-32H grown in human C8166 T cells showed a significant increase in the proportion (95%) and

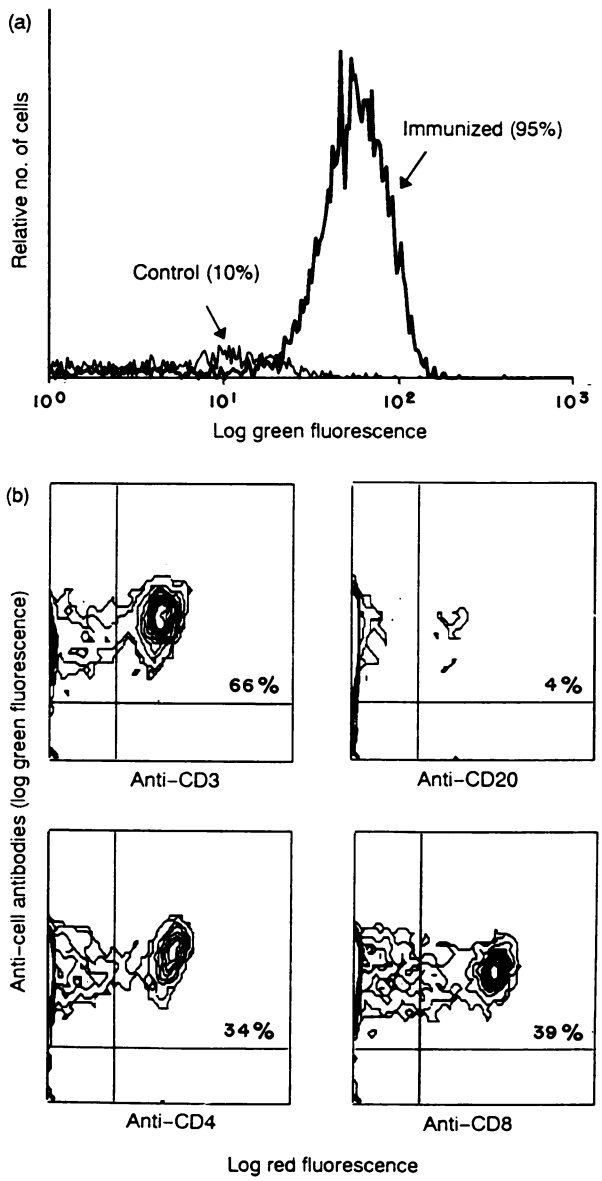

Figure 1. Flow cytometry analysis of the binding of macaque sera to human PMBC. (a) Relative binding of unimmunized control sera compared with sera from macaques immunized with C8166 grown SIV mac251. (b) Double fluorescence flow cytometry analysis of binding of anti-cell antibodies to four lymphocyte subsets.

intensity of fluorescence of human PBMC by flow cytometry, when compared with control (unimmunized) serum (10%) (Fig. 1a). Double fluorescence flow cytometry revealed that the leucocytes reacting with sera from immunized macaques were $CD3^+$, $CD4^+$ or $CD8^+$ T cells, B cells and monocytes (Fig. 1b). Macaque sera showed 39–66% reacting with $CD3^+$ cells, 34–40% with $CD4^+$ cells, 18–39% with $CD8^+$ cells, 4–9% with B cells and about 20% with monocytes (Fig. 1b). These results suggest that the macaque sera reacted with cell surface antigens common to T-cell subsets, B cells and monocytes.

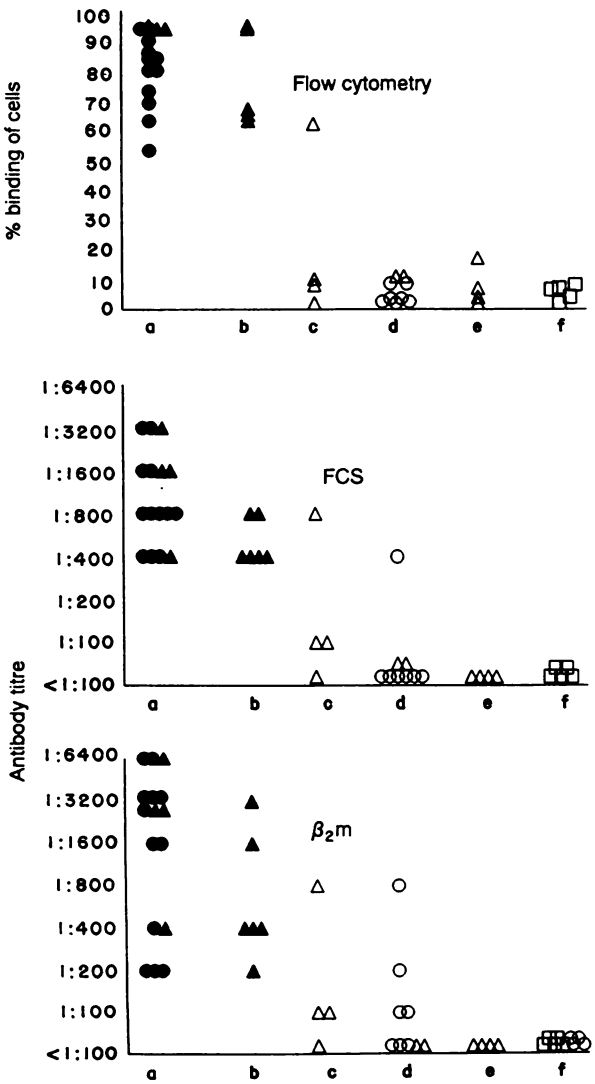

Figure 2. Serum anti-cell antibodies determined by flow cytometry of human peripheral blood lymphocytes and antibody titres determined by ELISA to FCS and human β_2m. (a) Sera from protected rhesus (●) or cynomolgus (▲) macaques immunized with cell-free SIV or with fixed infected C8166 cells. (b) Sera from protected cynomolgus macaques (▲) immunized with fixed uninfected C8166 cells. (c) Sera from infected cynomolgus macaques (△) immunized with fixed uninfected C8166 cells. (d) Sera from infected macaques (○, △) not immunized. (e) Sera from infected macaques (△) immunized with RK13 cells. (f) Preimmunization sera from untested macaques.

We then examined by flow cytometry 39 sera for anti-cell antibodies (Fig. 2). All 16 sera from rhesus or cynomolgus macaques immunized and protected with either cell-free virus or fixed infected C8166 cells showed high proportions of human PBMC reacting with the sera (53–95·5%) (Fig. 2a). However, sera from macaques immunized with fixed uninfected C8166 cells were clearly clustered in two groups. Those yielding a high proportion of binding (62–95·5%) were protected in six out of seven macaques, whereas three macaques with low binding (0–10·2%) were infected when challenged with SIV (Fig. 2b and c). Sera from unimmunized macaques also showed very low proportions of binding PBMC (0–11·1%), as did four macaques immunized with uninfected rabbit RK13 cells (0–17·4%; Fig. 2d and e). All unimmunized and RK13-immunized macaques were infected when challenged with SIV. The five preimmunization sera showed low reaction with the PBMC (2–8%). A very significant difference was found using the χ^2-test (with Yate's correction for small numbers) in anti-cell antibodies, determined by flow cytometry between the protected and infected macaques, which were immunized with the inactivated SIV vaccine or C8166 cells ($\chi^2 = 12{\cdot}028$, $P < 0{\cdot}001$, $n = 26$).

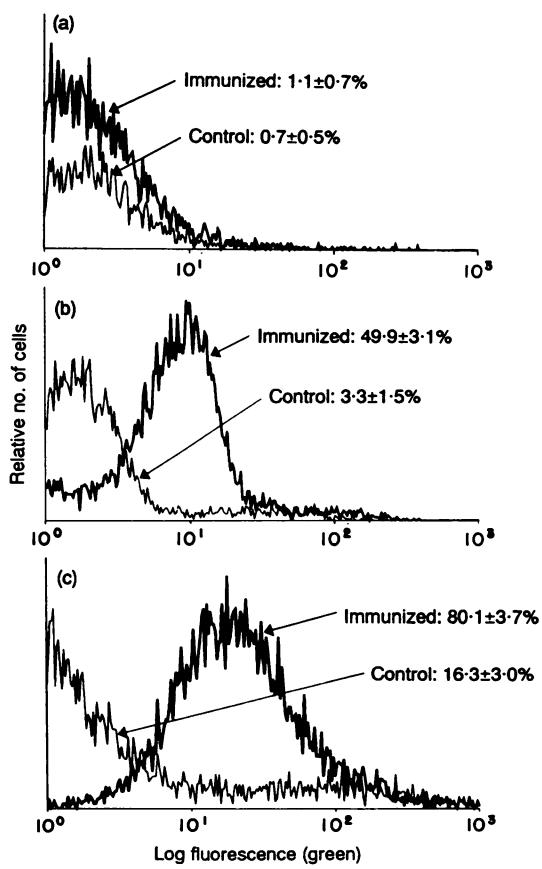

Figure 3. Flow cytometry of macaque peripheral blood lymphocytes (a) before ($n = 8$), (b) after incubation in RPMI containing 10% FCS ($n = 8$) and (c) after stimulation with Con A ($n = 6$). The cells were treated with sera from SIVmac unimmunized and control monkeys; mean ± SEM % are given.

Flow cytometry of macaque cells

Resting cells from many individual macaques failed to react with sera from immunized macaques (mean of 1·1 ± SEM 0·7%, $n = 8$). However, if the simian PBMC were incubated overnight in RPMI and 10% FCS, the sera from protected macaques reacted with 49·9 ± 3·1% cells (range of 36·8–64·6%), compared with 3·3 ± 1·5% (0–10·7%) of cells reacting with sera from unprotected macaques (Fig. 3a and b). Incubation with 10% autologous serum failed to increase the binding of simian PBMC (mean of 6·6 ± 3·2%; range of 2·4–13·0%; data not presented). An alternative approach was to activate macaque cells with a mitogen (Con A) for 48 hr, in the absence of FCS (Fig. 3c). Indeed, the proportion of activated cells reacting with the sera from protected macaques increased from 1·3 ± 0·8% (0–5·6%) to 80·1 ± 3·7% (67·3–91·3%), compared with sera from unprotected macaques, from 0·7 ± 0·5% (0·0–3·8) to 16·3 ± 3·0% (9·9–23·8%).

Antibodies to FCS

The findings that macaque cells will react with the antisera when treated with FCS led us first to consider the possibility that one or more of the FCS proteins may have induced the immune response. Significant anti-FCS antibodies (> 1:100) were found by ELISA in all 16 protected macaques which were immunized with the SIV vaccine either the cell-free partially purified virus or fixed infected C8166 cells (1:400–1:3200; Fig. 2a). Levels of (1:400–1:800) anti-FCS antibodies were also detected in all six protected macaques immunized with the uninfected C8166 cells alone; only one of four C8166-immunized unprotected animals yielded a significant anti-FCS level (1:800) (Fig. 2b and c). The nine unimmunized macaques and the four immunized with rabbit RK13 cells were all infected when challenged by SIV; only one of these 13 control sera gave a significant antibody titre to FCS (1:400) (Fig. 2d). None of the five preimmunization macaque sera yielded any anti-FCS antibodies. The χ^2-test (with Yate's correction) showed a significant difference in FCS antibodies ($\chi^2 = 12{\cdot}028$, $P < 0{\cdot}001$, $n = 26$), when protected and infected macaques were analysed in the SIV-inactivated and C8166 cell-immunized animals.

Adsorption studies of sera from macaques immunized with uninfected or SIV-infected C8166 T cells showed a dose-dependent absorption of anti-cell antibodies, requiring 2·4% and 1·7% FCS, respectively for 50% absorption (Fig. 4a). No absorption was recorded with BSA-coated beads. Competitive inhibition of anti-cell antibodies with anti-FCS also showed significant dose-dependent inhibition by flow cytometry (data not presented). These results suggested that antibodies were raised in macaques to some component of FCS which was used in growing the human T cells for SIV.

Antibodies to β_2m

We have examined the hypothesis that bovine β_2m in FCS in which the human T cells were grown may have exchanged with human β_2m in HLA class I antigen.[20] We examined the sera for antibodies to bovine β_2m by ELISA and found that 13 of 13 SIV-immunized macaques yielded significant antibodies (1:200–1:3200) but only two of six C8166-immunized and protected animals showed anti-β_2m antibodies (1:200 and 1:1600) (data not presented). The remaining four unprotected macaques in the latter group gave no significant anti-bovine β2m antibodies and this was also found with sera of eight control unimmunized and four RK13-immunized macaques. When we examined the sera for anti-human β_2m antibodies, all 16 sera from the protected SIV-immunized macaques gave significant antibodies (1:200–1:6400) (Fig. 2a). Unlike bovine β_2m antibodies, all six protected macaques immunized with the C8166 cells alone yielded β_2m antibodies (1:200–1:3200), whereas only one of four of the unprotected animals showed a significant antibody level (Fig. 2b and c). Among the 13 control sera all but two failed to show anti-β_2m antibodies > 1:100. A significant difference in human β_2m antibodies was found between the protected and infected macaques ($\chi^2 = 12{\cdot}028$, $P < 0{\cdot}01$, $n = 26$) in those immunized with the cell-free inactivated SIV or fixed C8166 cells. Furthermore,

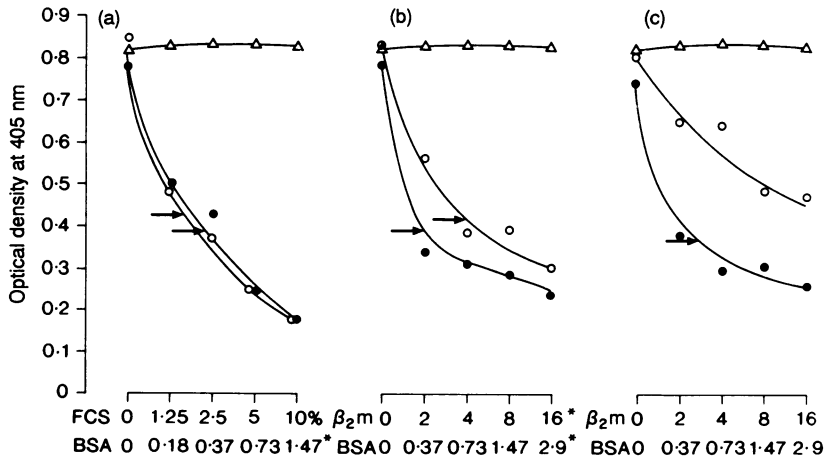

Figure 4. Adsorption studies of serum from an immunized and protected macaque with (a) FCS, (b) human β_2m, (c) bovine β_2m; these were assayed against uninfected (●), SIV-infected (○) C8166 cell preparations; BSA was used as a control antigen with uninfected C8166 cells (△). Arrows indicate 50% inhibition. *μM × 10^{-1}.

absorption studies showed that 50% absorption of antibodies by the uninfected C8166 T cells required 0·2 μM of human β_2m or 0·4 μM if tested against SIV-infected T cells (Fig. 4). Parallel studies with bovine β_2m demonstrated that 0·28 μM was required for 50% absorption of antibodies to the uninfected cells, but greater than 1·6 μM when tested against SIV-infected cells. No absorption was found with BSA. As β_2m is a highly conserved molecule, showing 77% homology between human and bovine β_2m (computer analysis of the sequences held on the SwissProt database), it is not surprising that antibodies were detected to both human and bovine β_2m.

Immunoprecipitation of human cells

Immunoprecipitation of surface-labelled human PBMC membranes with anti-human β_2m or HLA class 1 antibodies showed two major bands with M_r of about 12000 and 43000 which correspond to the M_r of the light and heavy chains of HLA class I molecules, respectively (Fig. 5a, lanes 4 and 5). Precipitation with sera from the vaccinated macaques (Fig. 5a, lane 2) gave rise to a faint band at M_r 43000 and a prominant band at M_r 1000; these are likely to be the α and β chains of HLA class I molecule. Anti-HLA class II antibodies failed to show any bands (Fig. 5a, lane 6), though this antibody reacted with Con A-activated cells and FCS-treated macaque cells (Fig. 5c, d, lane 6). Anti-bovine β_2m antibody precipitated a M_r 12000 band and a faint M_r 43000 was also detected, although the bands were much weaker when compared to anti-human β_2m (data not presented). Precipitation with anti-FCS prior to that of the vaccinate serum eliminated the M_r 12000 and to a lesser extent 43000 M_r band (Fig. 5a, lanes 7 and 2). Similarly, pretreatment with anti-β_2m antiserum followed by the vaccinate serum greatly reduced the M_r 12000 and 43000 bands (Fig. 5a, lanes 8 and 2). These results are consistent with the presence of anti-human HLA class I heavy and light chain antibodies in the sera from immunized and protected macaques, which may cross-react with antibodies to FCS. Finding anti-β_2m and anti-heavy chain antibodies to HLA class I agrees with the reports that these antigens are found in virus[10,15] and in HIV grown in human T-cell lines.[11] Although class II antibodies were not detected against resting human PBMC, class II was precipitated from the C8166 cell line used to grow the virus (data not presented).

Immunoprecipitation of macaque cells

Macaque cells were immunoprecipitated, using untreated cells, FCS-treated cells and Con A-activated cells (Fig. 5b–d). The M_r 12000 β_2m band was absent in the untreated cells, but appeared on treatment with FCS or Con A activation, when immunoprecipitated with the control and vaccinated monkey sera (Fig. 5b–d, lanes 1 and 2). However, the M_r 43000 band

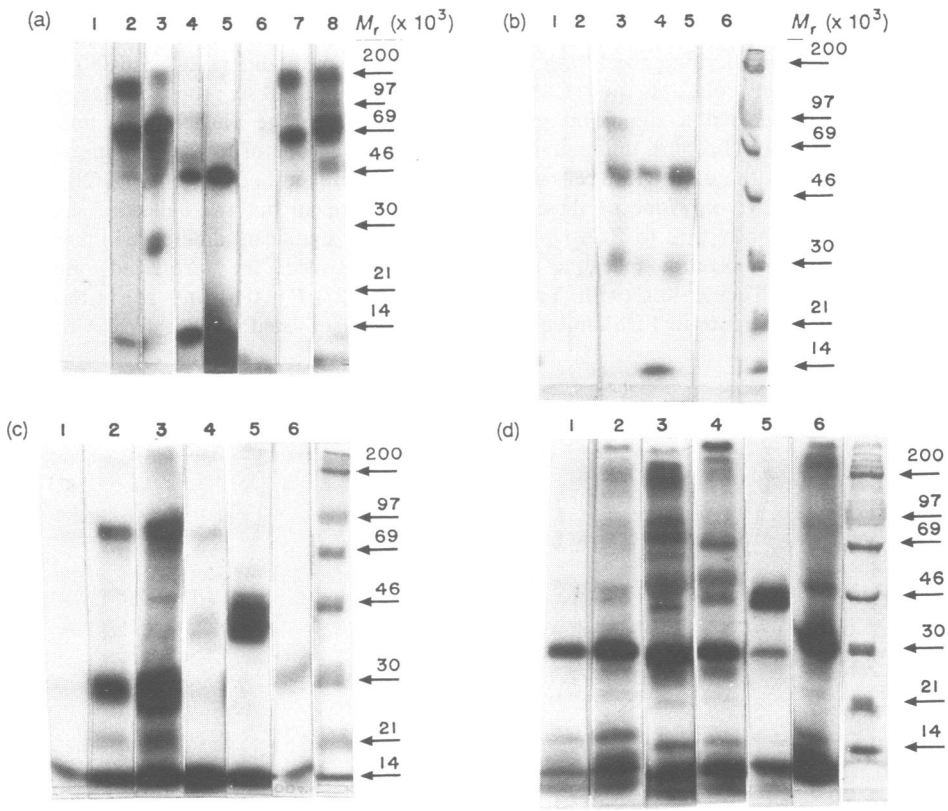

Figure 5. Immunoprecipitation of (a) resting human PBMC; (b) resting macaque PBMC; (c) FCS-treated macaque PBMC; (d) Con A-stimulated PBMC with the following antibodies: lane 1, macaque unimmunized control serum; lane 2, macaque immunized and protective serum; lane 3, rabbit anti-FCS; lane 4, anti-human class I (W632) mAb; lane 5, rabbit anti-human β_2m antibodies; lane 6, anti-human class II (DR) mAb; lane 7, anti-FCS followed by SIV vaccinate serum; lane 8, anti-human β_2m followed by SIV vaccinate serum.

was detected with the vaccinate serum only in the Con A activated cells (Fig. 5d, lane 2). These findings were similar to those observed by immunoprecipitation of human cells with vaccinate serum (Fig. 5a, lane 2). A pronounced M_r 30 000 was found with the vaccinate serum of the FCS- and Con A-treated macaque cells (Fig. 5c and d, lanes 2), and with the control serum of Con A-activated cells (Fig. 5d, lane 1). A similar M_r 30 000 band was revealed with human cells (Fig. 5a, lane 3) and macaque cells (Fig. 5b–d, lanes 3) precipitated with anti-FCS antiserum or anti-class II antibodies (Fig. 5d, lane 6 and to a lesser extent 5c lane 6). This raises the possibility that the M_r 30 000 band might constitute MHC class II antigen and needs further investigation. A M_r 65 000 band immunoprecipitated with the vaccinate and anti-FCS antibodies of human cells (Fig. 5a, lane 3) was also found with the Con A-treated macaque cells (Fig. 5d, lane 3). A prominent M_r 180 000 band immunoprecipitated with human cells (Fig. 5a, lane 2) was found only as a faint band with the Con A-treated macaque cells (Fig. 5d, lane 2). A significant feature of FCS and Con A treatment of macaque cells was the enhancement of bands immunoprecipitated with all the antisera used (Fig. 5c and d) and predictably the appearance of a number of new bands with the Con A-treated cells.

Antibodies to CD4

We have examined the sera from macaques for anti-CD4 antibodies by ELISA (titres range from 1:100 to 1:1600). Although many of the immunized macaques showed anti-CD4 antibodies, the 5% level of significance was not reached (data not shown). However, we have confirmed anti-CD4 antibodies by Western blots which showed a band with an apparent size of about M_r 45 000 with anti-CD4 (Leu-3A), by the serum from a macaque immunized with partially purified SIV (a representative serum from 10 sera), rabbit anti-FCS and rabbit anti-human β2 m serum but not with normal rabbit serum (Fig. 6). Indeed, rabbit anti-FCS antiserum showed a titre of 1:12 800 against CD4 by ELISA, as compared with no detectable antibodies (< 1:50) with normal rabbit serum. Competitive inhibition of anti-CD4 antibodies (OKT4) with anti-FCS or anti-cell antibodies showed significant inhibition by flow cytometry (data not presented). These results suggest that CD4 antibodies are induced by immunization with human T cells grown in FCS.

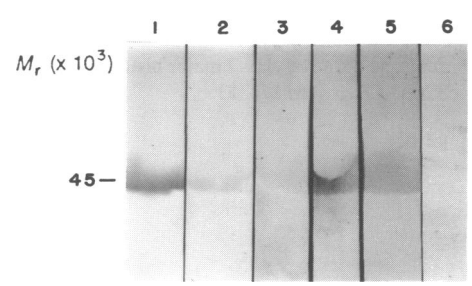

Figure 6. Western blot analysis of binding to recombinant CD4 antigen (10 μg/lane) with the following antisera: lane 1, OKT4 control; lane 2, serum from animals immunized and protected with partially purified SIV; lane 3, macaque unimmunized; lane 4, rabbit anti-FCS anti-serum; lane 5, rabbit anti-human β_2m antiserum; lane 6, normal rabbit serum.

DISCUSSION

These results are consistent with the reports that antibodies to human cell surface antigens are elicited by immunization with inactivated SIV grown in human T cells or by the uninfected T cells alone.[13–16] There was a significant association between protection from infection with live SIV and the anti-cell antibodies. Characterization of the cell subsets revealed that the antibodies reacted with cell surface antigens common to T cells (CD4 and CD8) B cells and monocytes. This is consistent with the findings that class I heavy and light chain (β_2m) antibodies are detected within the anti-cell antibodies,[11–14,16] as these are common to T and B cells and monocytes. Indeed, a significant association between antibodies to human β_2m and protection from SIV infection was established.

Previous attempts to detect significant anti-cell antibodies to rhesus or cynomolgus PBMC were unsuccessful. This was unsatisfactory, as any protective effect of these antibodies to human cell surface antigens had to be demonstrable against cross-reacting macaque cells. We were surprised to find that resting macaque PBMC failed to react with sera from vaccinated macaques, unless the cells were treated with FCS or activated with a mitogen. Indeed, treatment of macaque cells with FCS not only enabled the vaccinate sera to react with them, but also revealed heavy and light chain class I antibodies on immunoprecipitation which was not evident with resting cells or cells similarly treated with autologous serum. The involvement of FCS was enhanced by finding a significant relationship between antibodies to FCS and protection from SIV infection. Inhibition studies and immunoprecipitation with human and FCS-treated macaque cells are consistent with a role of FCS in the protective immune response against SIV infection. Reprecipitation of FCS antibody-treated cell membranes with anti-cell antibodies resulted in loss or decrease in bands with M_r 12 000 and 43 000, which were identified as the HLA class I light and heavy chains. Bovine β_2m may be one of the components in FCS which may enhance the immune response of human T cells in macaques, as it readily exchanges with human β_2m in HLA class I antigen.[20] If SIV activity incorporates class I antigens in its envelope during budding the β_2m component might be of bovine origin. In the group immunized with cell-free partially purified and cell-associated virus this may constitute a greater quantitative component of bovine β_2m (13 of 13 positive by ELISA) than that associated with uninfected cells (two of six positive by ELISA).

Rabbit anti-FCS antiserum also reacted with CD4 by ELISA (1:12 800) and this raised the possibility that in addition to HLA class I antibodies, CD4 antibodies may have been induced during immunization with the vaccine. Indeed, we have found low levels of anti-CD4 antibodies by ELISA with sera from animals immunized with partially purified SIV and infected cells (titres 1:100–1:1600). Furthermore, a band of about M_r 45 000 was detected with anti-CD4, serum from macaques immunized and protected with partially purified SIV and rabbit anti-FCS, but not with normal macaque or rabbit serum by Western blots. These results indicate that CD4 antibodies are induced by immunization with human T cells grown in FCS. We suggest that in addition to the involvement of HLA class I antibodies, CD4 antibodies may prevent SIV from binding to $CD4^+$ cells. Indeed, anti-CD4 antibodies have been generated by immunizing macaques with recombinant

human CD4, resulting in significant inability to recover virus after infection with live SIV.[21] There is also evidence that mitogenic activation of lymphocytes reveals a hidden MHC class I α chain epitope which becomes accessible to a cross-reacting mAb which also inhibits syncytium formation.[26] We have preliminary evidence that lymphocytes from macaques immunized with heat-inactivated, uninfected or SIV-infected C8166 T cells yield significant proliferative responses when stimulated with FCS (stimulation indices of 4·7–9·0). This raises the possibility that FCS may activate macaque lymphocytes, to enable a hidden MHC class I α chain to bind with anti-cell antibodies which inhibits syncytium formation of HIV-infected cells.[26] The results of this investigation are consistent with the findings that human cellular antigens and FCS in the culture medium in which SIV is grown may influence the immunogenicity of the vaccine.[9,10] We suggest that human cellular components augmented by FCS elicit anti-class I heavy chain, β_2m, CD4 and FCS antibodies which are responsible for protection against SIV infection in macaques.

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