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Antigen presentation in the sheep: generation of antigen-specific T-cell lines

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SUMMARY

Antigen-specific sheep T-cell lines have been generated *in vitro* from peripheral blood mononuclear cells (PBMC). PBMC prepared from antigen-primed animals were cultured in the presence of ovalbumin (OVA) or purified protein derivative (PPD). After 5 days of culture, activated antigen-specific cells were expanded by further culture in the presence of recombinant human interleukin-2 (IL-2). Cell lines generated after two cycles of antigen stimulation followed by expansion with IL-2 show a proliferative response to antigen only in the presence of autologous antigen-presenting cells (APC) and recognize only the antigen used in the original stimulation. An OVA-specific cell line was found to be capable of recognizing a synthetic peptide corresponding to amino acid residues 323–338 of OVA. The cell lines also responded by proliferation in an allogeneic mixed leucocyte reaction (MLR). Cell-surface phenotyping shows that the cell lines comprise both CD4- and CD8-positive cells.

INTRODUCTION

Considerable progress has been made in recent years in understanding how T cells recognize soluble protein antigen in association with MHC molecules (Schwartz, 1985; Buus, Sette & Grey, 1987). A major contribution to this progress has been the generation and use of antigen-specific T-cell lines and T-cell clones (Gillis & Smith, 1977). It is now known that following antigen recognition, T cells secrete interleukin-2 (IL-2) and express high-affinity IL-2 receptors at their cell surface. The combination of IL-2 with its receptor and subsequent signal transduction allows antigen-stimulated T cells to undergo clonal expansion (Smith, 1984). With this knowledge, antigen-specific T cells may be enriched for *in vitro* by repeated cycles of antigen stimulation followed by expansion with IL-2. These T cells may be maintained as a polyclonal population or, following cloning, as cells with a unique antigen specificity. Recognition of antigen by T cells may then be assayed either by lymphokine release (Kappler *et al.*, 1980) or by T-cell proliferation (Seeger & Oppenheim, 1970).

It has also become clear that a variety of cell types may present antigen to primed T cells. However, only a limited number of accessory cells appear capable of stimulating a primary T-cell proliferative response (Inaba & Steinman, 1984).

Abbreviations: APC, antigen-presenting cell; BSA, bovine serum albumin; Con A, concanavalin A; DMSO, dimethyl sulphoxide; FITC, fluorescein isothiocyanate; HPLC, high-pressure liquid chromatography; MHC, major histocompatibility complex; MLR, mixed leucocyte reaction; OVA, ovalbumin; PPD, purified protein derivative.

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We are interested in studying aspects of antigen presentation using afferent and efferent cells collected by cannulation of sheep lymphatic vessels. Both lymphocyte compartments contain MHC class II-positive cells. In particular, afferent lymph contains a population of MHC class II-positive dendritic cells which might be involved in antigen carriage to the lymph node (Hoefsmit, Duijvestijn & Kamperdijk, 1982). To study the accessory potential of afferent and efferent cells requires antigen-specific T-cell lines to defined antigens. This paper describes the generation of sheep antigen-specific T-cell lines, which is a first step in studying accessory cell function in sheep.

MATERIALS AND METHODS

Antigens

Ovalbumin (OVA) (Grade V, Cat. no. A5503) was obtained from Sigma Chemical Company Ltd (Poole, Dorset). Purified protein derivative of bovine tuberculin, (PPD, batch 291) was obtained from the Central Veterinary Laboratory, Weybridge, Surrey. OVA peptide 323–328 was synthesized on a CRB Pepsynthesiser II (Cambridge Research Biochemicals Ltd, Cambridge) using fluorenylmethoxycarbonyl (Fmoc)-polyamide chemistry (Dryland & Sheppard, 1986). The pentafluorophenyl esters of Fmoc-derivatized amino acids, incorporating *t*-Butyl based side-chain blocking groups, and Pepsyn KA polyamide support resin were obtained from Cambridge Research Biochemicals Ltd, Cambridge. The Fmoc protecting groups were removed by treatment with 20% piperidine with final deprotection and cleavage from the resin carried out with trifluoroacetic acid. The peptide was purified by reverse-phase HPLC using a C_{18} Zorbax column (Dupont, Delaware, U.S.A.)

then freeze-dried. Foot and mouth disease virus (FMDV strain A24) peptide 141-160 was a generous gift from Dr T. Dole (Institute for Animal Health, Pirbright, Surrey). Antigens were made to 1 mg/ml in culture medium, sterilized by 0·22 μm filtration and stored at 4°.

Immunization of animals

Finnish Landrace sheep, 1–3 years old, purchased from the Moredun Research Institute, Edinburgh, were immunized with 1 mg OVA injected over two sites intramuscularly in a 1-ml mixture of phosphate-buffered saline (PBS) and complete Freund's adjuvant containing H3TRA *Mycobacterium tuberculosis* (Sigma, cat. no. F4258). Sheep were also immunized with five human doses *Bacillus Calmet–Guerin* (BCG; Glaxo Laboratories, Greenford, Middlesex) in two sites intradermally.

Medium

RPMI-1640 (cat no. 074-1800, Gibco Biocult, Uxbridge) was supplemented with 2 mM L-glutamine, 100 U/ml benzyl penicillin and 100 U/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol and 2 g/l sodium bicarbonate. Culture medium consisted of RPMI-1640 with supplements and 10% fetal calf serum (FCS) (Flow Laboratories, Irvine, Ayrshire).

Interleukin-2

Recombinant human IL-2 was a generous gift from Biogen SA (Geneva, Switzerland).

Generation of antigen-specific cell lines

Cell lines were generated as described by Lanzavecchia, Parodi & Celada (1983). Briefly, PBMC from antigen-primed sheep were resuspended at 2×10^6/ml in 24-well plates with culture medium containing an optimum concentration of antigen. After 5–7 days incubation at 37°, blasts were harvested over Lymphoprep (Nyegaard, Oslo, Norway) and cultured at 1×10^5/ml in medium containing 120 pM recombinant human IL-2. Cells were cultured for 14 days with fresh additions of IL-2 every 3–4 days. At the end of this period viable cells were harvested over Lymphoprep and re-stimulated with antigen by culturing 4×10^5 resting cells with 4×10^6 X-ray irradiated autologous PBMC. After 5–7 days blasts were harvested and again expanded with IL-2. After this second cycle of antigen and IL-2 expansion, viable blasts were harvested over Lymphoprep and resuspended for cryopreservation at 5×10^6/ml in an ice-cold mixture of 75% fetal calf serum, 15% RPMI and 10% DMSO. Cells were frozen in 1-ml glass ampules using a programmed cell-freezer (Planar Products Ltd, London) then stored under liquid nitrogen.

Generation of Con A blasts

Sheep PBMC prepared by centrifugation of defibrinated peripheral venous blood on Lymphoprep were resuspended at 2×10^6/ml in culture medium containing 5 μg/ml Con A (cat. no. C-2010, Sigma) and cultured at 37° for 4 days. Viable blasts were harvested over Lymphoprep, washed three times and frozen for storage under liquid nitrogen as described above.

Collection of lymph cells

Efferent cells were collected by cannulation of the prefemoral lymph node as described by Hall & Morris (1962). Afferent cells were obtained by cannulation of a psuedo-afferent lymphatic vessel. This was performed as described for the prefemoral efferent lymphatic except that the node had been excised at least 8 weeks earlier (Hopkins *et al.*, 1985). Cells were stored under liquid nitrogen as described above.

Recovery of frozen cells

Cells were rapidly thawed at 37° and placed on ice. To 1 ml of cells, 0·2 ml of ice-cold culture medium was added every 30 seconds for 2·5 min. This was repeated with 0·4 ml and then 0·8 ml of culture medium. Cells were washed three times with ice-cold culture medium using the minimum of centrifugation and pipetting.

Antigen-specific proliferation assay

Resting antigen-specific T cells were cultured in 96-well flat-bottomed micro-culture plates in a final volume of 200 μl. Triplicate cultures were stimulated where appropriate with the indicated doses of antigen in the presence or absence of X-ray irradiated autologous PBMC. Cultures were incubated at 37° in a humidified atmosphere of 5% CO_2/95% air for 5 days. Cells were then pulsed with 1 μCi[^{3}H]thymidine (specific activity 2Ci/mM; Amersham, Amersham, Bucks) and 5 hr later collected onto glass filter paper using a semi-automated harvester. [^{3}H]thymidine incorporation was assessed by liquid scintillation counting. The data is expressed as the geometric mean for the triplicate cultures and the standard deviation was generally less than 10% of the mean.

IL-2 proliferation assay

Blast cells recovered from liquid nitrogen were resuspended at 3×10^5/ml and dispensed in 100-μl aliquots. Triplicate two-fold dilutions of recombinant IL-2 were added in 100 μl volumes and the cultures incubated for 3 days. Proliferation was measured by the uptake of [^{3}H]thymidine over the last 5 hr of culture.

Antibodies

SBU-T1 (Mackay *et al.*, 1985), SBU-T4 and SBU-T8 (Maddox, Mackay & Brandon, 1985) are mouse monoclonal antibodies that recognize the sheep cell surface glycoproteins CD5, CD4 and CD8, respectively. These were a generous gift from Dr M. Brandon (University of Melbourne, Australia). VPM8 is a mouse monoclonal antibody generated in our laboratory that has specificity for sheep immunoglobulin light chain (P. Jones, personal communication). Antibodies were used in culture supernatant form.

Indirect immunofluorescence

Cell surface phenotype was assessed using aliquots of 1×10^6 cells washed with PBS containing 1% bovine serum albumin (BSA) and 0·1% sodium azide. Cells were incubated with 50 μl of monoclonal antibody supernatant for 30 min on ice. After three washes, 50 μl of FITC-conjugated sheep anti-mouse IgG were added for 30 min on ice. Cells were washed three times, resuspended in 100 μl PBS and fixed by the addition of an equal volume of 1% paraformaldehyde. Staining was analysed by flow cytometry using a Becton-Dickenson FACS IV.

RESULTS

Four sheep were used in these experiments. Cell lines were generated from all four animals: a total of six cell lines were produced, three specific for OVA and three specific for PPD.

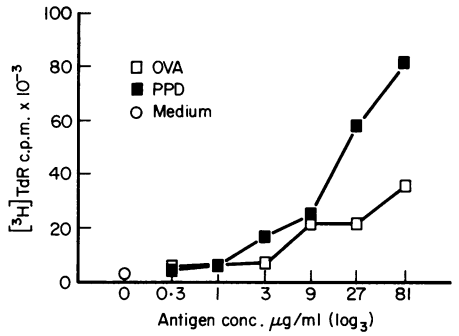

Figure 1. Proliferative response by PBMC to antigen. PBMC prepared from antigen-primed animals were cultured at 1×10^5/well with various dilutions of either ovalbumin or PPD. Proliferation was measured after 5 days of culture by [^{3}H]thymidine uptake over the last 5 hr.

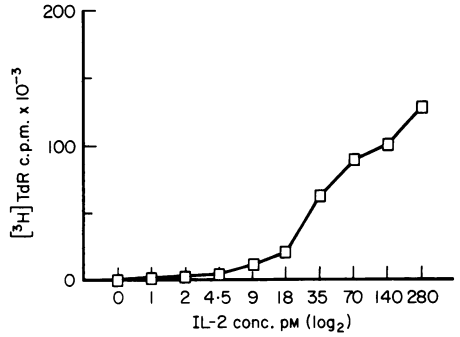

Figure 2. Response of sheep Con A blasts to human IL-2. Con A blasts were cultured at 3×10^4/well with various dilutions of recombinant human IL-2. Proliferation was measured after 3 days of culture by [^{3}H]thymidine uptake over the last 5 hr.

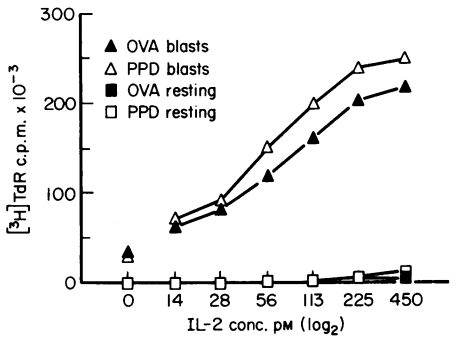

Figure 3. Response of activated and resting cell lines to human IL-2. Antigen-specific cell lines harvested after antigen stimulation or following expansion with IL-2 were cultured at 3×10^4/well with various dilutions of human recombinant IL-2. Proliferation was measured after 3 days of culture by [^{3}H]thymidine uptake over the last 5 hr.

The results described below are typical of those obtained for all the cells lines.

Proliferative response of PBMC to soluble protein antigen

Figure 1 shows the results of a typical response by sheep PBMC to antigen in a secondary *in vitro* proliferative assay. Animals

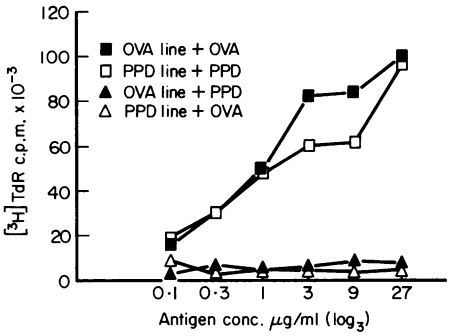

Figure 4. Antigen-specificity of cell lines. Antigen-specific cells were harvested after two cycles of antigen-stimulation and expansion with IL-2. Cells were cultured at 5×10^4/well in the absence (triangles) or in the presence of 5×10^4/well X-ray irradiated autologous PBMC (squares) with various dilutions of antigen. Proliferation was measured after 5 days of culture by [^{3}H]thymidine uptake over the last 5 hr. The response by cell lines cultured in the absence of antigen either with or without irradiated autologous PBMC was < 2000 c.p.m.

Table 1. Cell-surface phenotype of antigen-specific cell lines

	% positive cells		
Surface marker	1	2	3
CD5	77	78	73
CD4	66	56	53
CD8	24	33	28
Ig	6	8	10

Three different antigen-specific cell lines (two PPD-specific and one OVA-specific) were stained with either SBU-T1, SBU-T4, SBU-T8 or VPM8 monoclonal antibodies followed by sheep anti-mouse immunoglobulin-FITC. Cells were fixed with 1% paraformaldehyde and analysed by flow cytometry. The data shows the percentage of positive cells staining.

primed with the live vaccine BCG and OVA show a response to PPD and OVA, respectively, in the range of 1–100 μg/ml of antigen. The response to PPD is usually of greater magnitude than that stimulated by OVA. The most consistent and reproducible results from the proliferative assay were obtained with medium containing 2-mercaptoethanol and supplemented with FCS. Higher stimulation indices could be obtained using medium supplemented with heat-inactivated lamb serum but this medium did not support proliferation of all PBMC tested (data not shown).

Response of sheep cells to recombinant human IL-2

It has become clear that the successful culture of T cells *in vitro* requires IL-2. Figure 2 shows that sheep Con A blasts respond

OVA 323–338	Ile Ser Asn Ala Val [His Ala Ala His] Ala Glu Ile Asn Glu Ala Gly
FMD A24 141–160	Ser Gly Arg — Arg Gly Asp Met Gly Ser Leu Ala Ala [Arg Val Val Lys] Gln Leu Pro

Figure 5. Synthetic peptides containing T-cell epitopes. Amino acid sequences of OVA 323–328 (McReynolds *et al.*, 1978) and FMD A24 peptide 141–160 (Makoff *et al.*, 1982). The hatched areas enclose a motif of amino acids found in several immunogenic peptides (Rothbard 1986).

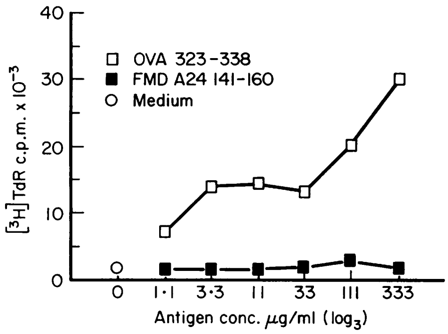

Figure 6. Response to OVA peptide 323–338. OVA-specific cells were cultured at 5×10^4/well in the presence of 5×10^4/well X-ray irradiated autologous PBMC with various dilutions of synthetic peptides. Proliferation was measured after 5 days of culture by [^{3}H]thymidine uptake over the last 5 hr.

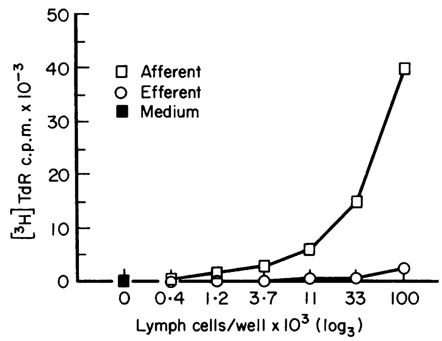

Figure 7. Allogeneic response stimulated by lymph cells. PPD-specific T cells were cultured at 5×10^4/well in the presence of 5×10^4/well X-ray irradiated allogeneic afferent or efferent cells, collected from the same animal, in round-bottomed microculture plates. Proliferation was measured after 5 days of culture by [^{3}H]thymidine uptake over the last 5 hr.

to recombinant human IL-2. A response is seen to all the doses of IL-2 tested. Recombinant human IL-2 is effective in the pico molar range, suggesting it is stimulating high-affinity IL-2 receptors (Robb, Greene & Rusk, 1984). Routinely, 120 pM recombinant human IL-2 were used to expand activated antigen-specific cells.

Generation of antigen-specific cell lines

Antigen-specific blast cells were generated by stimulation of PBMC with antigen. Activated cells were subsequently expanded with recombinant human IL-2 and allowed to return to the resting phase during a 14-day culture. Figure 3 shows that antigen-specific blasts, like Con A blasts, proliferate in response to all the concentrations of recombinant human IL-2 tested. Resting cells, harvested at least 4 days after the last addition of IL-2, do not show a significant response to the doses of IL-2 tested. This shows indirectly that activation of T cells by antigen-presenting cells leads to the transient expression of functional IL-2 receptors (Cantrell & Smith, 1983).

Figure 4 shows the specificity of resting cell lines to antigen presented by irradiated autologous PBMC. Both cell lines show a proliferative response to their specific antigen at all doses tested. In contrast, PBMC from which these lines were derived showed a response to both antigens. The response by cell lines cultured with antigen in the absence of accessory cells was the same as T cells cultured alone.

Table 1 shows the cell-surface phenotype of three representative cell lines generated from different sheep. The lines comprise predominately $CD5^+$ T cells and include $CD4^+$ and $CD8^+$ cells. Not all of the cells from the lines are distinguished by either SBU-T1 or VPM8 antibodies. It is possible that these lines contain null cells, those which do not express detectable CD5 antigen or surface immunoglobulin. Such cells are reported to represent up to 25% of sheep peripheral blood (MacKay *et al.*, 1988).

Response to synthetic peptides

It is known that some T-cell clones recognize peptides of the original stimulating antigen. This has led to the development of predictions for T-cell epitopes that are based on amino acid sequence analysis. (DeLisi & Berzofsky, 1985; Rothbard, 1986). Figure 5 shows the amino acid sequence of OVA peptide 323–338 that is immunogenic in H-2^d mice (Shimonkevitz *et al.*, 1984). Figure 6 shows that a sheep T-cell line specific for OVA contains clones that respond to this peptide. A response is seen to all doses of the OVA peptide tested. This response is not seen on the absence of antigen-presenting cells. The cell line shows no response to the control peptide FMD A24 141-160.

Response to allogeneic lymph cells

T cells may also recognize and respond by proliferation to MHC class II molecules expressed on allogeneic cells (Alter & Bach, 1970). Figure 7 shows the response by a PPD-specific cell line to allogeneic afferent and efferent lymph cells, collected from the same animal. Afferent lymph cells are capable of stimulating a proliferative response at all the numbers of cells tested. In contrast, efferent lymph cells only stimulate a significant

response at the highest number of cells tested. Efferent lymph cells collected from three different animals all stimulate a response that is less in magnitude than afferent cells.

DISCUSSION

We are investigating antigen presentation by cells collected from cannulated sheep afferent and efferent lymphatic vessels. Here, we have described the development of a T-cell read-out system to assess the presentation of antigen by MHC class II-positive cells. We have successfully used a strategy of serial antigenic stimulation followed by expansion with IL-2 to generate antigen-specific sheep T-cell lines. These lines have been generated from PBMC, obtained from antigen-primed animals, which show a response to more than one antigen. After two cycles of antigen stimulation and expansion with IL-2, cell lines are generated that respond to only the original stimulating antigen and do so only in the presence of autologous APCs. Our success in generating these lines has been helped by the use of a defined source of IL-2, namely recombinant human IL-2. We have since found that activated sheep T cells may respond equally well to recombinant IL-2 of either human or bovine origin (R. Bujdoso, unpublished observations).

It has become clear that processing of soluble protein antigen by APCs includes denaturation and proteolytic degradation. T-cell clones have been identified that recognize partially denatured myoglobin (Allen & Unanue 1984; Streicher *et al.*, 1984) and some that recognize peptides of OVA (Shimonkevitz *et al.*, 1983; Watts *et al.*, 1984). We have shown that a sheep polyclonal OVA-specific T-cell line is capable of responding to OVA peptide 323–338. This peptide displays two properties that have recently been proposed to predict T-cell epitopes. Firstly, the peptide is an amphiphathic alpha helix, that is one portion of the molecule is hydrophobic and the other hydrophilic (DeLisli & Berzofsky, 1985; Watts *et al.*, 1985). Secondly, this peptide contains a motif of amino acids found in several immunogenic peptides (Rothbard, 1986) which have been implicated as containing amino acid residues involved in binding to both the T-cell receptor and MHC class II (Sette *et al.*, 1987). Whichever of these two properties best predicts immunogenic peptides, our observation suggests that predictions made in other species concerning putative T-cell epitopes will be applicable to the sheep system.

A second feature of antigen recognition by these T-cell lines is their ability to respond to allogeneic lymph cells. Both afferent and efferent lymph compartments contain cells which express MHC class II molecules and therefore have the potential to stimulate this response. However, afferent lymph also contains a population of class II-positive dendritic cells that comprise 1–5% of the total population. We believe that the greater allogeneic response stimulated by afferent cells is due to the presence of these dendritic cells (R. Bujdoso, unpublished observations). Our result is in agreement with other reported experiments that suggest lymphocytes, in particular resting B cells, are poor stimulators of T-cell proliferation in a primary response to antigen (Inaba & Steinman, 1984).

Our ability to generate specific cellular reagents in the sheep will allow us to study unique populations of lymph cells with respect to their role in antigen presentation. These cell lines are currently being used to study the properties of afferent lymph dendritic cells and these results will be reported elsewhere.

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REFERENCES

ALLEN P.M. & UNANUE E.R. (1984) Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. *J. Immunol.* **132,** 1077.

ALTER B.J. & BACH F.H. (1970) Lymphocyte reactivity *in vitro*. I. Cellular reconstitution of purified lymphocyte response. *Cell Immunol.* **1,** 207.

BUUS S., SETTE A. & GREY H.M. (1987) The interaction between protein derived immunogenic peptides and Ia. *Immunol. Rev.* **98,** 115.

CANTRELL D.A. & SMITH K.A. (1983) Transient expression of interleukin-2 receptors. Consequences for T cell growth. *J. exp. Med.* **158,** 1895.

DELISI C. & BERZOFSKY J.A. (1985) T-cell antigenic sites tend to be amphipathic structures. *Proc. natl. Acad. Sci. U.S.A.* **82,** 7048.

DRYLAND A. & SHEPPARD R.C. (1986) Peptide synthesis. Part 8. A system for solid-phase synthesis under low pressure continuous flow conditions. *J. Chem. Soc. Perkin Trans.* **1,** 125.

GILLIS S. & SMITH K.A. (1977) Long-term culture of tumor-specific cytotoxic T cells. *Nature (Lond.)*, **268,** 154.

HALL J.G. & MORRIS B. (1962) The output of cells in lymph from the popliteal node of sheep. *Q. J. exp. Physiol.* **47,** 360.

HOEFSMIT E.C.M., DUIJVESTIJN A.M. & KAMPERDIJK E.W.A. (1982) Relation between Langerhan's cells, veiled cells and interdigitating cells. *Immunobiology*, **161,** 255.

HOPKINS J., MCCONNELL I., BUJDOSO R. & MUNRO A.J. (1985) Studies on MHC Class II products on sheep peripheral and efferent lymph cells. In: *Immune Responses in Foetal and Adult Sheep* (eds B. Morris and M. Miyasaka), p. 442. Editiones, Roche, Basel.

INABA K. & STEINMAN R. (1984) Resting and sensitised T lymphocytes exhibit distinct stimulatory (antigen-presenting cell) requirements for growth and lymphokine release. *J. exp. Med.* **160,** 1717.

KAPPLER J.W., SKIDMORE B., WHITE J. & MARRACK P. (1980) Antigen-inducible H-2-restricted, interleukin-2-producing T cell hybridomas: Lack of independent antigen and H-2 recognition. *J. exp. Med.* **153,** 1198.

LANZAVECCHIA A., PARODI B. & CELADA F. (1983) Activation of human B lymphocytes: frequency of antigen-specific B cells triggered by alloreactive or by antigen-specific T cell clones. *Eur. J. Immunol.* **13,** 733.

MACKAY C.R., KIMPTON W.G., BRANDON M.R. & CAHILL R.N.P. (1988) Lymphocyte subsets show marked differences in their distribution between blood, and afferent and efferent lymph draining peripheral lymph nodes. *J. exp. Med.* **167,** 1755.

MACKAY C.R., MADDOX J.F., GOGOLIN-EWENS K.J. & BRANDON M.R. (1985) Characterization of two lymphocyte differentiation antigens, SBU-T1 and SBU-T6. *Immunology* **55,** 729.

MCREYNOLDS L., O'MALLEY B.W., NISBET A.D., FOTHERGILL J.E., GIVOL D., FIELDS S., ROBERTSON M. & BROWNLEE G.G. (1978) Sequence of chicken ovalbumin mRNA. *Nature (Lond.)*, **273,** 723.

MADDOX J.F., MACKAY C.R. & BRANDON M.R. (1985) Surface antigens, SBU-T4 and SBU-T8, of sheep T lymphocyte subsets defined by monoclonal antibodies. *Immunology*, **55,** 739.

MAKOFF A.J., PAYNTER C.A., ROWLANDS D.J. & BOOTHROYD J.C. (1982) Comparison of the amino acid sequence of the major immunogens from the three serotypes of foot and mouth disease virus. *Nucleic Acid. Res.* **10,** 8285.

ROBB R.J., GREENE W.C. & RUSK C.M. (1984) Low and high affinity cellular receptors for interleukin 2. *J. exp. Med.* **160,** 1126.

ROTHBARD J.B. (1986) Peptides and the cellular immune response. *Ann. Inst. Past. Virol.* **137E,** 518.

SCHWARTZ R.H. (1985) T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. *Ann. Rev. Immunol.* **3,** 237.

SEEGER R.C. & OPPENHEIM J.J. (1970) Synergistic interaction of macrophages and lymphocytes in antigen-induced transformation of lymphocytes. *J. exp. Med.* **132,** 44.

SETTE A., BUUS S., COLON S., SMITH J.A., MILES C. & GREY H.M. (1987) Structural characteristics of an antigen required for its interaction with Ia and recognition by T cells. *Nature* (*Lond.*), **328,** 395.

SHIMONKEVITZ R., COLON S., KAPPLER J.W., MARRACK P. & GREY H.M. (1984) Antigen recognition by H-2-restricted T cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen. *J. Immunol.* **133,** 2067.

SHIMONKEVITZ R., KAPPLER J., MARRACK P. & GREY H.M. (1983) Antigen recognition by H-2 restricted T cells. I. Cell-free antigen processing. *J. exp. Med.* **158,** 303.

SMITH K.A. (1984) Interleukin 2. *Ann. Rev. Immunol.* **2,** 319.

STREICHER H.Z., BERKOWER I.J., BUSCH M., GURD F.R.N. & BERZOFSKY J.A. (1984) Antigen conformation determines processing requirements for T-cell activation. *Proc. natl. Acad. Sci. U.S.A.* **81,** 6831.

WATTS T.H., BRIAN A.A., KAPPLER J.W., MARRACK P. & MCCONNELL H.M. (1984) Antigen presentation by supported planar membranes containing affinity-purified I-A^d. *Proc. natl. Acad. Sci. U.S.A.* **81,** 7564.

WATTS T.H., GARIÉPY J., SCHOOLNIK G.K. & MCCONNELL H.M. (1985) T-cell activation by peptide antigen: Effect of peptide sequence and method of antigen presentation. *Proc. natl. Acad. Sci. U.S.A.* **82,** 5480.