can become visible if, as in mice, the right virus isolate infects humans of the correct general genetic background so that HLA-regulation of the T cell response becomes recognizable.

The presented results and interpretations lead to another important consequence, with respect to application of immune modulating therapeutic protocols used during immunopathologically mediated disease. Unless one knows the balance, kinetics and actual relationship between parasite and host response very precisely, it is equally likely that one will influence the host/parasite balance in a beneficial or a determinal direction. In conclusion, the proposal is made, illustrated and supported by experimental evidence that T cell-mediated immunopathology triggered initially by low- or non-cytopathic infectious agents may cause diseases, susceptibility to which is linked to the MHC. It is obvious that not all MHC-disease associations are explained by this pathophysiological mechanism, but a reasonable guess would be that many of such linkages may follow the outlined rules. Conversely, the proposal implies that MHC-disease associations quite generally signal T cell-mediated pathophysiology of the disease.

1 This summary is an updated version of the paper given on the occasion of the Paul Ehrlich Prize ceremonies in 1983; it was also presented at the meeting 'New Trends in Allergy II' in München 1985, and is reproduced here with the permission of Springer Verlag, Heidelberg.

2 Paul, W. E., and Benacerraf, B., Science *195* (1977) 1293.
3 Townsend, A. R. M., and McMichael, A. J., Progr. Allergy *36* (1984) 10.
4 Zinkernagel, R. M., and Doherty, P. C., Adv. Immun. *27* (1979) 51.
5 Dausset, J., and Svejgaard, A., HLA and Disease Munksgaard, Copenhagen 1977.
6 Doherty, P. C., and Zinkernagel, R. M., Transplant. Rev. *19* (1974) 89.
7 Möller, G., Immun. Rev. *70* (1983).
8 McDevitt, H. O., and Bodmer, W. F., Lancet *1* (1974) 1269.
9 Cerottini, J. C., and Brunner, K. T., Adv. Immun, *19* (1974) 67.
10 Zinkernagel, R. M., Pfau, C. J., Hengartner, H., and Althage, A., Nature *316* (1985) 814.
11 Zinkernagel, R. M., and Althage, A., J. exp. Med. *145* (1977) 644.
12 Byrne, J. A., and Oldstone, M. B. A., J. Virol. *51* (1974) 682.
13 Mims, C. A., Pathogenesis of Infectious Disease, 2nd edn. Academic Press, London 1982.
14 Fenner, F., McAuslan, B. R., Mims, C. A., Sambrook, J., and White, D. O., The Biology of Animal Viruses, 2nd edn. Academic Press, London 1974.
15 Lehmann-Grube, F., Virol. Monogr. *10* (1971) 2.
16 Rowe, W. R., Res. Rep. nav. med. Res. Inst. *12* (1954) 167.
17 Cole, G. A., Nathanson, N., and Prendergast, R. A., Nature *238* (1972) 335.
18 Mackay, I. R., Bull. N.Y. Acad. med. Sci. *52* (1976) 433.
19 Jeannet, M., and Farquet, J. J., Lancet *2* (1974) 1383..
20 Bang, F. B., Adv. Virus Res. *23* (1973) 269.
21 Friedman, R. M., and Vogel, S. N., Adv. Immun. *34* (1983) 97.
22 Allen, J. E., and Doherty, P. C., Immunogenetics *21* (1985) 581.
23 Pfau, C. J., Valenti, J. K., Pevear, D. C., and Hunt, K. D., J. expl Med. *156* (1982) 79.
24 Wiktor, T. J., and Koprowski, H., Proc. natn. Acad. Sci. USA *75* (1984) 3938.
25 Cohen, D., and Dausset, J., Progr. Immun. *5* (1973) 1.
26 Biddison, W. E., Ward, F. E., Shearer, G. M., and Shaw, S., J. Immun. *124* (1980) 548.

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Virus-immune T cells and the major histocompatibility complex: Evolution of some basic concepts over the past two years

by P. C. Doherty

Department of Experimental Pathology, John Curtin School of Medical Research, Canberra ACT 2601 (Australia)

Key words. T cells; major histocompatibility complex; cell-mediated immunity; immune response genes; influenza.

The lecture that I gave at the University of Frankfurt as part of the proceedings associated with the award of the Paul Ehrlich-Ludwig Darmstaeder Prize in March 1983 was not prepared in a format that was suitable for publication. My understanding of the subject has been summarized in two recent reviews that are readily accessible[1,2]. There would be little point in repeating the exercise here. However, on re-reading the outline of the 1983 lecture, it was obvious that a number of the ideas current then must now be considered to be rather dated. The present, short account, thus concentrates on the way that our understanding of major histocompatibility complex (MHC)-restricted T cell recognition of virus-infected cells has been modified over the past two years. Much of this change reflects the development of T cell clones and the application of molecular biology. Many of the speculations about the nature of molecular interactions that were raised by cell-biology experiments have been, or are in the process of being, resolved. Other problems appear to be more intractable.

The T cell receptor

The debate about whether or not the T cell had two polymorphic receptors[3] for virus and MHC glycoprotein, or one receptor which recognizes some complex (or interaction product) of virus and MHC[4], was very much alive in 1983 and is still not buried. Some of us have long been of the opinion that only the one-receptor model can explain the results of various biological experiments[5-7], though we have tried to explore conceptually the ways in which a two receptor model might operate[8,9].

Much of the current evidence[10] seems to indicate that a single, clonotypic T cell receptor is central to MHC-restricted T cell recognition. This receptor consists of covalently-linked α and β chains, which are immunoglobulin

(Ig)-like but have diverged from the immunoglobulin genes before, for instance, speciation to mouse and man. Most published evidence to date is concerned with the β chain, which may only have about 15–30 variables (V)-region genes, though the spectrum of receptor specificities may be increased by using different reading frames from the diversity (D)-region[10–12].

Some α chain sequences are also published[13], but there is little information yet available on the range of V-regions for the α chain[10]. Proposals that the T cell receptor genes may be linked to those coding for the MHC glycoproteins, or for Ig heavy chain, have been shown to be incorrect[13,14]. The α chain genes of mouse and man are both located on chromosome 14, while the β chain genes are on chromosome 7 (man) or chromosome 6 (mouse)[14–17].

Earlier speculations[18–21] on thymic learning of both self-tolerance and MHC-restriction specificity are also likely to be considerably modified in the not too distant future. The groups working with the mouse[22–24] are in agreement that T cell receptors (or functional re-arrangements of α and β chain genes coding for the receptors) can be found on thymocytes as early as day 17 of gestation, and are present on all major adult thymocyte populations. Many immature thymocytes also express rearranged γ and β chain, but not α chain, genes: the γ gene[25,26] is similar to α and β and could act as an additional T cell receptor subunit. However mRNA for γ is at very low levels in mature T cells, so it may be that γ is involved principally in intrathymic differentiation pathways[24,27]. The new information on the clonotypic receptor, together with accumulating evidence concerning the progressive expression of other T cell surface molecules[28–30], such as Lyt2, L3T4 and the I1–2 receptor will obviously contribute to the development of a better understanding of thymic differentiation[31,32].

Though it does seem likely that the 'one-receptor' model for immune recognition of self+x will prevail, the nature of the MHC-restricted T cell repertoire is still far from clear. Some questions, such as the relative roles of germline genes and somatic variants[33], can obviously be answered by the molecular approach. However, at this stage, there is no way of distinguishing DNA coding for the clonotypic receptors of helper/DTH and cytotoxic T cells[34], even though the patterns of MHC restriction may be different. The answer to this problem may lie elsewhere.

MHC glycoproteins and targeting of functional T cells

We proposed that the evolutionary basis of the MHC-restriction patterns for cytotoxic (class I) and helper (class II) T cells reflected a biological need to focus lymphocytes with different functions into appropriate anatomical niches[5,35]. Class I MHC glycoproteins are found on most cells throughout the body, though the levels in brain may be very low and some neurons seem to be irrevocably negative[36–38]. The class II MHC molecules tend to be expressed on cells involved in the generation and regulation of immune responses: B cells, monocyte/macrophages dendritic cells and other T cells[39–41]. The idea was that the class I MHC-restricted, Lyt2$^+$ (in mouse) T8$^+$ (in man) virus-immune cytotoxic T lymphocytes (CTL) could operate to clear virus-infected targets from any tissue, while the Lyt2$^-$, L3T4 (in mouse) T8-T4$^+$ (in man) helper T cells[42] would be targeted into lymphoid organs where growth factors secreted at short range should act to promote the immune response in both the T and B cell compartments[43]. Delayed-type hypersensitivity (DTH) T cells, that are also class II-restricted would be recruited into inflammatory lesions by recognizing macrophages that had localized to such sites. These propositions may still be useful as generalizations for explaining in vivo phenomena, but it is obvious that they cannot (together with other concepts derived from them) be regarded as any sort of invariant dogma.

The exceptions to the postulate that CTL are recognizing class I MHC+neoantigen (x), while DTH/helper T cells are specific for class II MHC+x, have come from studies with T cell clones. There are now a number of instances where virus-immune CTL in man have been shown to be T8-T4$^+$ and class II MHC-restricted[44,45]. This may not necessarily be the case for alloreactive CTL directed at class II MHC determinants: at least some T cells in this category are T4-T8$^+$, or Lyt2$^+$[46,47]. The suggestion has thus been made that the T4 (or L3T4 in the mouse)[42] molecule in some way participates in the binding event between the self-restricted T cell, whether helper or CTL, and class II MHC glycoproteins expressed on the target[48,49]. Similarly, T8 and Lyt2 may be involved in a direct interaction with a constant domain on the class I MHC molecule. Perhaps the alloreactive CTL are of sufficiently high affinity that they may not require this additional binding event[46,47]. At this stage, it does seem likely that the Lyt (or T4-T8) phenotype of MHC-restricted T cells generally defines the MHC-restriction pattern, not the functional status, of T lymphocytes.

These observations also effectively dispose of the idea that the MHC glycoproteins act essentially as 'signalling' channels, with the 'signal' for lysis being directed via the class I molecules and that for the promotion of help functioning via the class II molecules. In fact, it may not be necessary for the CTL to recognize any MHC glycoprotein at all. According to the experiments of Kranz et al.[50] MHC-negative targets coated with a monoclonal anti-idiotype antibody specific for the clonotypic receptor of a CTL clone are lysed by the effectors in question. The MHC glycoproteins may also not be required for lectin-dependent T cell-mediated cytotoxicity[51]. Furthermore, cells can be lyzed by CTL which are focussed onto cell surface by hybrid antibodies, one arm of which binds to the T cell receptor while the other recognizes a non-MHC target antigen[52]. The implication is thus that the only function that the MHC glycoprotein on the target fulfills is to be recognized in association with neoantigen by a single, clonotypic T cell receptor. This is basically the 'altered self' (or self+x) hypothesis that we proposed some years back to explain MHC-restricted cytotoxicity[4,5]. I have recently discussed some of the conceptual difficulties that arise with this model of T cell receptor specificity at length, but with no great clarity, elsewhere[53].

The molecule that does seem to have some signalling function in the T cell target interaction is the T3 phosphoprotein, which is loosely associated with the human T cell receptor[10,49]. Binding of monoclonal antibody (MAb) to T3 results in a rapid increase of intracellular Ca^{2+} in the T

cell[54]. Furthermore, the MAb-T3 interaction can induce both cytotoxic and non-cytotoxic (in some cases) T cell clones to display antigen non-specific cytotoxic activity[55]. The latter observation may go some way towards explaining the numerous reports[55-57] of degeneracy, or modification of specificity, of T cell clones that are maintained in vitro. Also, a virus-specific, class II MHC-restricted proliferating T cell clone has been shown to become cytotoxic after prolonged in vitro culture[58]. Thus, at this stage, the only molecular entity known to have a direct signalling role to play in T cell function is associated with the plasma membrane of the lymphocyte, not the target.

The other dogma that has been discarded is that the production of lymphokines is a property unique to class II MHC-restricted T cells. Many class I MHC-restricted CTL clones produce γ interferon[59,60]. The growth-promoting lymphokine, Il-2, is made mostly by class II MHC-restricted T cells, but a subset of class I restricted lymphocytes also secrete relatively low levels of Il-2[60]. Perhaps this may explain why we were able to stimulate class I-restricted, primary virus-immune CTL responses in the absence of any class II homology between the irradiated stimulator environment and negatively-selected (for alloreactivity) T cells[61]. This flew in the face of the then current thinking about the need for T-T help[3], which may be demonstrated in some in vitro situations[62]. At this stage the need, or otherwise, for class II MHC-restricted T cell help in primary virus-immune CTL responses in vivo is still not clear[63-65].

The existence of class II MHC-restricted CTL raises important questions concerning the nature of immune regulation[66]. Both resting and activated B cells have been shown to present antigen in association with class II MHC glycoproteins[67,68]. The current model is that binding of antigen (or anti-Ig) to the Ig receptor on the B cell results in cross-linking, membrane depolarization and enhanced Ia expression[69]. In addition, the antigen-Ig complex is thought to be interiorized, disassociated and the antigen (or a fragment of it) re-presented on cell surface in the context of self Ia[70]. There would thus seem to be the potential for the selective elimination of responder B cells by class II MHC restricted CTL. The possibility that such effectors may cause immunopathology has also been raised by Jacobsen et al.[71], who isolated measles-virus specific, class II-restricted CTL from a patient with multiple sclerosis (MS): the T cells were HLA-DR2 restricted, presence of DR2 being a known risk factor in MS. Perhaps, except in immunopathological conditions, Ia-restricted CTL are essentially a phenomenon of in vitro T cell cloning procedures. It would not be surprising if those T cells which constitutively produce high levels of Il-2 are more readily cloned.

The generalization that the Lyt2$^+$ (OKT8$^+$) CTL tend to be concerned with class I MHC glycoproteins, while the L3T4$^+$ (OKT4$^+$) helper-inducers that produce growth factors (such as Il-2) are targeted onto the class II molecules may still be of value when considering normal host recovery mechanisms in vivo. However, these associations between function, Lyt or OKT phenotype and MHC-restriction specificity are not invariant, and a number of exceptions have been found with cloned T cell lines. The one thing that is now clear is that the class I and class II MHC-glycoproteins cannot be thought of as differiantial 'signalling channels' for the selective delivery of a lytic (class I) or inductive (class II) message from the T cell. Effector function is a property of the physiological capacity of the individual T lymphocyte, not the particular target MHC glycoprotein.

Specificity of influenza-immune cytotoxic T cells

The question of the nature of T cell specificity for virus-coded determinants assumed immediate significance following the discovery of MHC restriction for virus-immune CTL[4,5]. Could we show specificity for particular virus-coded determinants or were T cells recognizing some form of modified MHC molecules[4,5], a speculation which was in accord with Jerne's earlier model for the thymus?[72] The influenza A viruses seemed to offer a ready made biological system for analyzing this specificity question[73].

These RNA viruses have a segmented genome, with 8 segments that can assort independently when two virus strains are grown together in the same cell[73]. The major viral surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (N), can thus be associated differentially with each other and with the genes coding for the nucleoprotein (NP), matrix (M_1 and M_2) proteins and the viral polymerases. The HA and N glycoproteins vary considerably between the different influenza A viruses, being subjected to antibody-mediated selective pressures, while the NP and M components tend to be relatively stable[74].

The initial finding was that mice primed with, for instance, an HA1N1 influenza A virus generated CTL populations which were lytic for MHC-compatible targets infected with an HA3N2 strain, even though antibodies to these two viruses did not cross-neutralize[75-77]. Furthermore, mice which had been exposed to HA1N1 showed a classical secondary CTL response when infected later with HA3N2[78]. The dominance of the cross-reactive CTL response, which was decidedly unfashionable in the context of then current thinking about immunity in influenza, was disputed for a time[79] but was soon confirmed for both man[80,81] and the rat[82]. In fact, it seems that influenza infection in adult humans generally procedes in the context of a secondary CTL response which may have some protective value[83]. In the mouse, limiting dilution analysis has shown quite clearly that 80–90% of CTL clones are of the cross-reactive type[84,85].

The question of what exactly it is that the influenza immune CTL are seeing on cell surface is still not resolved. However, the identities of the virus-coded determinants that are required for T cell recognition are rapidly being established. This has depended on the availability of T cell clones, and on gene transfection protocols which employ either SV40 genes or vaccinia virus as expression vectors.

Many of the cross-reactive (e.g. between HA1N1 and HA3N2) CTL appear to be directed at a product of the viral NP gene[86-88]. Target L cells transfected with the NP gene are lyzed to a high level by MHC-compatible bulk CTL populations, generated following priming with a variety of influenza A viruses. It should be noted that NP is not a glycoprotein, and does not have the character-

istics of an integral membrane protein. Although NP may be found on the surface of virus-infected cells[89] little, if any, could be detected serologically on the transfected L cells[87]. This follows the general principle that the antigenic determinants recognized by antibody are not those that are of interest to CTL[1,2], though there are exceptions to this rule[90].

More recently, another cross-reactive virus-coded determinant has been found on cell-surface in considerable quantities[91]. This is the M_2 protein, which is detected only in infected cells and not in virus particles[92]. It now seems that M_2 is an integral membrane protein with 18 NH_2-terminal amino acids experessed on cell surface: ten of these are conserved for all strains of influenza A virus that have been sequenced so far[91]. It would not be surprising if the M_2 molecule turns out to be a major virus-coded target molecule of interest to the cross-reactive CTL population.

A small proportion (probably ‹10%) of CTL clones are specific for the viral HA molecule, as established by gene transfection protocols[87,93,94]. Some of these T cells may be cross-reactive for HA1 and HA2, but not for HA3[87], reflecting the extent of molecular identity. However, even though the HA molecule is the predominant virus-coded entity on cell surface, it does not seem to be particularly immunogenic for the CTL compartment.

The findings with the influenza A viruses thus have two lessons for us. The first is the general point that, though a molecule may be expressed in high concentrations on the cell plasma membrane, it may be of relatively little significance in, at least, the cytotoxic arm of the CMI response. In fact, a variety of 'internal' components such as NP and even viral polymerase gene products[95] must be considered as targets for T cell recognition. The realization that this caveat exists may be of particular importance for the interpretation of tumor immunology[1]. The second point is that strategies for producing influenza vaccines directed solely at the viral HA and N may be ill-conceived. Perhaps it is also necessary to incorporate viral components that may be involved in the induction of CMI[83], though such vaccines would have to be designed so that the relevant antigenic determinants are presented on cell surface in an immunogenic configuration.

Class I MHC immune response genes in virus infections

The experiments with influenza virus, and with vaccinia virus, led to the realization that examples of low CTL responsiveness mapping to different class I MHC glycoproteins show two distinct patterns[96,97]. The first is that there may be a total immune response (Ir) gene defect associated with a particular MHC allele, which does not vary with any combination of MHC genes in recombinant or F1 mice. A case in point is H-2D^k-vaccinia virus[97]. We thought that the same situation might apply for H-2K^b-influenza virus[96], but later found that responder T cells were present at relatively low frequency and were more dependent on added helper factors (in limiting dilution cultures) than were those asociated with, for instance, H-2K^k[98]. The second situation involves the apparent immunodominance of some MHC alleles. An instance is that H-2D^b is associated with quite a strong CTL response to influenza virus or vaccinia virus when complemented with H-2K^b, but is a weak Ir gene when H-2K^k is also present[96,97,99].

The debate as to whether total non-responder situations reflect a failure to form an appropriate anatomical nexus between viral and MHC components, or are a consequence of there being 'holes' in the T cell repertoire due to cross-reactivity with self components, continues and is likely to continue for some time[1,99-101]. A reasonable, but essentially unhelpful, statement would seem to be that failure to respond reflects the lack of an 'appropriate' antigenic configuration, where 'appropriateness' is determined by the available spectrum of T cell receptors.

We do know something about the antigenic regions of both MHC and non-MHC molecules that are required for CTL recognition[1,2]. 'Exon-shuffling' protocols with MHC genes transfected into L cells indicate that, in most (though not all) cases, both the α_1 and α_2 domains of class I MHC molecules are required for CTL recognition, which presumably means that the CTL are generally interested in conformational determinants expressed on the more accessible, outer regions of the MHC glycoproteins[102]. The α_3 domain, which is adjacent to the cell plasma membrane, does not seem to be of major significance in determining antigenicity.

With the SV40 system, where the large T antigen is recognized by CTL[103,104], there is evidence that recognition by individual CTL clones restricted to the same class I MHC gene product may variously require expression of either the amino or the carboxy terminal region of the T molecule[105]. There is also a preliminary report[106] that different segments of the influenza NP gene product are of interest to H-2^b-and H-2^k-restricted CTL. These findings can be interpreted as reflecting varied patterns of co-stabilization of viral and class I MHC components[5], as we have discussed previously at some length[1]. Such interactions may obviously determine one half of the Ir-gene equation.

The immunodominance situation may prove much more difficult to analyze[99]. Perhaps antigenic competition for key reactive sites is involved: evidence in support of this idea has been found for class II MHC Ir genes[107]. However, there are problems in applying this model directly to the class I MHC immunodominance hierarchies described to date. In the mouse lymphocytic choriomeningitis virus (LCMV) model, for instance, H-2L^d is absolutely dominant in the H-2^d (K^{d}D^{d}L^d) haplotype: no CTL response is found for H-2K^d or H-2D^d[108]. However, if H-2L^d is not present, CTL are generated in association with H-2k^d, though it is a weaker Ir gene than H-2L^d. Furthermore, these H-2K^d-restricted CTL lyse virus-infected K^{d}D^{d}L^d targets. Thus, if antigenic competition between K^d and L^d for relevant viral molecules were to be invoked as an explanation for the immunodominance of L^d, it would be necessary to argue that the requirements for T cell stimulation are much more demanding than those for lysis. The possibility[100] that the need for self-tolerance to L^d results in the suppression of clones with the potential to recognize K^d+LCMV is obviously one avenue of investigation that must be explored. Even so, findings from previous experiments with the vaccinia model[99] would not offer cause for undue optimism. Understanding these Ir gene immunodominance hierarchies offers a considerable challenge.

Conclusions

My perceptions of the MHC restriction phenomenon, and the implications of MHC-restriction for T cell function, have been aired at length in several, recent reviews. Rather than cover the same ground again here, it seemed more appropriate to attempt to summarize progress during 1984 and 1985 in areas that are of particular interest to me because I was a member of research teams that generated the initial discoveries or concepts. These are: MHC-restriction of virus-immune CTL; the 'one-receptor-altered self (self+x)' model for T cell recognition; the cross-reactivity of the CTL response to cells infected with different influenza A viruses; and class I MHC Ir gene effects for virus-immune CTL.

In broad terms, those questions that are susceptible to the direct application of the molecular biology approach have shown enormous advances over this two-year period. The molecular nature of the T cell receptor is rapidly being resolved. The identities of viral genes that code for the entities recognized by influenza-immune CTL are being established, though it may be much more difficult to characterize the actual antigenic structure recognized by the T cell receptor. In addition, we now seem to be in a position to discard the idea that the MHC molecules themselves are signalling channels. Futhermore, a strong possibility has emerged that cell surface molecules other than the clonotypic receptor, such as those of the Lyt-OKT series, are involved in determining the spectrum of MHC-restricted interaction between T cell and target. This in no way diminishes the significance of the fact that we may be dealing with a single, clonotypic T cell receptor that binds to 'altered-self (self+x)'.

At this stage, though we have gained considerable insights into the identities of both the self and non-self determinants that are required for T cell recognition, the question of the nature of the actual T cell-target interaction at the molecular level may be much more difficult to solve. Other problems, such as the biological basis of the immunodominance hierarchies in class I MHC Ir gene effects, are also likely to remain with us for some considerable time. In addition, analysis of the roles of various T cell populations in disease states, and the interactions between immunity, immunopathology and autoreactivity, is likely to continue to yield new insights. Perhaps the study of host response in virus infections may still provide original perspectives on the central problem of our discipline: understanding the nexus between immunity, on the one hand, and self-tolerance, on the other.

1 Doherty, P.C., Knowles, B.B., and Wettstein, P.J., Adv. Cancer Res. *42* (1984) 1.
2 Doherty, P.C., Br. med. Bull. *41* (1985) 7.
3 Zinkernagel, R.M., Callahan, G.N., Althage, A., Cooper, J., Klein, P.A., and Klein, J., J. exp. Med. *147* (1978) 882.
4 Zinkernagel, R.M., and Doherty, P.C., Nature *251* (1974) 547.
5 Doherty, P.C., and Zinkernagel, R.M., Lancet *1* (1975) 1406.
6 Doherty, P.C., and Bennink, J.R., J. exp. Med. *149* (1979) 150.
7 Hurwitz, J.L., Pan, S., Wettstein, P.J., and Doherty, P.C., Immunogenetics *17* (1983) 79.
8 Doherty, P.C., Götze, D., Trinchieri, G., and Zinkernagel, R.M., Immunogenetics *3* (1976) 517.
9 Doherty, P.C., Effros, R.B., Bennink, J.R., and Gerhard, W., Perspect. Virol. *19* (1977) 73.
10 Marx, J.L., Science *227* (1985) 733.
11 Chien, Y-h., Gascoigne, N.R.J., Kavaler, J., Lee, N.E., and Davis, M.M., Nature *309* (1984) 322.
12 Yoshikai, Y., Anatoniou, D., Clark, S.P., Yanagi, Y., Sangster, R., Van den Elsen, P., Terhorst, C., and Mak, T.W., Nature *312* (1984) 521.
13 Kranz, D.M., Saito, H., Disteche, C.M., Swisshelm, K., Pravtcheva, D., Ruddle, F.H., Eisen, H.N., and Tonegawa, S., Science *227* (1985) 941.
14 Croce, C.M., Isobe, M., Palumbo, A., Puck, J., Ming, J., Tweardy, D., Erikson, J., Davis, M., and Rovera, G., Science *227* (1985) 1044.
15 Dembić, Z., Bannwarth, W., Taylor, B.A., and Steinmetz, M., Nature *314* (1985) 271.
16 Cullins, M.K.L., Goodfellow, P.N., Spurr, N.K., Solomon, E., Tanigawa, G., Tonegawa, S., and Owen, M.J., Nature *314* (1985) 273.
17 Caccia, N., Kronenberg, M., Saxa, D., Haars, R., Bruns, G.A.P., Goverman, G., Malissen, M., Willard, H., Yoshikai, Y., Simon, M., Hood, L., and Mak, T.W., Cell *37* (1984) 1091.
18 Zinkernagel, R.M., Immun. Rev. *42* (1978) 224.
19 Bevan, M.J., and Fink, P.J., Immun. Rev. *42* (1978) 4.
20 Doherty, P.C., and Bennink, J.R., Scand. J. Immun. *12* (1980) 271.
21 Doherty, P.C., Korngold, R., Schwartz, D.H., and Bennink, J.R., Immun. Rev. *58* (1981) 38.
22 Roehm, N., Herron, L., Cambier, J., DiGuisto, D., Haskins, K., Kappler, J., and Marrack, P., Cell *38* (1984) 577.
23 Snodgrass, H.R., Kisielow, P., Kiefer, M., Steinmetz, M., and von Boehmer, H., Nature *313* (1985) 592.
24 Raulet, D.H., Garmon, R.D., Saito, H., and Tonegawa, H., Nature *314* (1985) 103.
25 Saito, H., Kranz, D.M., Takogaki, Y., Hayday, A.C., Eisen, H.N., and Tonegawa, S., Nature *309* (1984) 757.
26 Kranz, D.M., Saito, H., Heller, M., Takagaki, Y., Haas, W., Eisen, H.N., and Tonegawa, S., Nature *313* (1985) 752.
27 Snodgrass, H.R., Dembić, Z., Steinmetz, M., and von Boehmer, H., Nature *315* (1985) 232.
28 Ceredig, Rh., Dialynas, D.P., Fitch, F.W., and MacDonald, H.R., J. exp. Med. *158* (1983) 1654.
29 Ceredig, Rh., Lowenthal, J.W., Nabholz, M., and MacDonald, H.R., Nature *314* (1985) 98–100.
30 Raulet, D.H., Nature *314* (1985) 101.
31 Trowbridge, I.S., Lesley, J., Trotter, J., and Hyman, H., Nature *315* (1985) 666.
32 Samelson, L.E., Lindsten, T., Foulkes, B.J., van den Elsen, P., Terhorst, C., Davis M.M., Germain, R.N., and Schwartz, R.H., Nature *315* (1985) 765.
33 Augustin, A.A., and Sim, G.K., Cell *39* (1984) 5.
34 Rupp, F., Acha-Orbea, H., Hengartner, H., Zinkernagel, R., and Joho, R., Nature *315* (1985) 425.
35 Zinkernagel, R.M., and Doherty, P.C., Adv. Immun. *27* (1979) 51.
36 Lampson, L.A., in: Monoclonal Antibodies and Functional Cell Lines, pp. 153–189. Eds R.H. Kennett, K.B. Bechtol and T.J. McKearn. Plenum Press, New York 1984.
37 Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., Battye, F., and Schrader, J.W., Nature *310* (1984) 688.
38 Doherty, P.C., Trends Neurosci. *8* (1985) 41.
39 Unanue, E.R., Beller, D.I., Lu, C.Y., and Allen, P.M., J. Immun. *132* (1984) 1.
40 Van Voorhis, W.C., Valinsky, J., Hoffman, E., Luban, J., Hair, L.S., and Steinman, R.M., J. exp. Med. *158* (1983) 174.
41 Singh, S.K., Abramson, E.J., Krco, C.J., and David, C.S., J. molec. cell. Immun. *1* (1984) 147.
42 Dialynas, D.P., Wilde, D.B., Marrack, P., Pierres, A., Wall, K.A., Havran, W., Otten, G., Loken, M.R., Pierres, M., Kappler, J., and Fitch, F.W., Immun. Rev. *74* (1983) 29.
43 Lowenthal, J.W., Zubler, R.H., Nahbolz, M., and MacDonald, H.R., Nature *315* (1985) 669.
44 Meuer, S.C., Hodgdon, J.C., Cooper, D.A., Hussey, R.E., Fitzgerald, K.A., Schlossman, S.F., and Reinherz, E.L., J. Immun. *131* (1983) 186.
45 Kaplan, D.R., Griffith, R., Braciale, V.L., and Braciale, T.J., Cell. Immun. *88* (1984) 193.
46 Spits, H., Ijssel, H., Thomson, A., and de Vries, J., J. Immun. *131* (1983) 678.
47 Vidovic, D., Juretic, A., Nagy, Z.A., and Klein, J., J. Immun. *11* (1981) 499.
48 Swain, S.L., Immun. Rev. *74* (1983) 129.
49 Reinherz, E.L., Meuer, S.C., and Schlossman, S.F., Immun. Rev. *74* (1983) 83.

50 Kranz, D.M., Tonegawa, S., and Eisen, H.N., Proc. natn. Acad. Sci. USA *81* (1984) 7922.
51 Bell, S.M., and Stern, P.L., Eur. J. Immun. *15* (1985) 59.
52 Staerz, U.D., Kanagawa, O., and Bevan, M.J., Nature *314* (1985) 628.
53 Doherty, P.C., in: Paradoxes in Immunology. Eds G. Hoffman, J. Levy and G. Nepom. CRC Press, Boca Raton in press (1985).
54 O'Flynn, K., Zanders, E.D., Lamb, J.R., Beverley, P.C.L., Wallace, D.L., Tatham, P.E.R., Tax, W.J.M., and Linch, D.C., Eur. J. Immun. *15* (1985) 7.
55 Spits, H., Yssel, H., Leeuwenberg, J., and de Vries, J.E., Eur. J. Immun. *15* (1985) 88.
56 Brooks, C.G., Urdal, D.L., and Henney, C.S., Immun. Rev. *72* (1983) 43.
57 Simon, M.M., Weltzien, H.U., Bühring, H.J., and Eichmann, K., Nature *308* (1984) 367.
58 Fleischer, B., Nature *308* (1984) 365.
59 Morris, A.G., Lin, Y.L., and Askonas, B.A., Nature *295* (1982) 150.
60 Kelso, A., and Glasebrook, A.L., J. Immun. *132* (1984) 2924.
61 Bennink, J.R., and Doherty, P.C., Nature *276* (1978) 829.
62 Bennink, J.R., Schwartz, D.H., and Doherty, P.C., Cell. Immun. *60* (1981) 347.
63 Allan, J.E., and Doherty, P.C., Cell. Immun. *90* (1985) 401.
64 Saron, M.F., Shidani, B., Guillon, J.-C., and Truffa-Bachi, P., Eur. J. Immun. *14* (1984) 1064.
65 Rouse, B.T., Miller, L.S., Turtinen, L., and Moore, R.N., J. Immun. *134* (1985) 926.
66 Janeway, C.A. Jr, Bottomly, K., Babich, J., Conrad, P., Connzen, S., Jones, B., Kaye, J., Katz, M., McVay, L., Murphy, D.B., and Tite, J., Immun. today *5* (1984) 99.
67 Chesnut, R.W., Colon, S.M., and Grey, H.M., J. Immun. *128* (1982) 1764.
69 Coggeshall, K.M., and Cambier, J.C., J. Immun. *134* (1985) 101.
70 Grey, H.M., and Chesnut, R., Immun. today *6* (1985) 101.
71 Jacobson, S., Nepom, G.T., Richert, J.R., Biddison, W.E., and McFarland, H.F., J. exp. Med. *161* (1985) 263.
72 Jerne, N.K., Eur. J. Immun. *1* 1971) 1.
73 Palese, P., and Kingsbury, D.W., Genetics of Influenza Viruses. Springer Verlag, Vienna 1985.
74 Mitchell, D.M., McMichael, A.J., and Lamb, J.R., Br. med. Bull. *41* (1985) 80–85.
75 Zweerink, H.J., Courtneidge, S.A., Skehel, J.J., Crumpton, M.J., and Askonas, B.A., Nature *267* (1977) 354.
76 Effros, R.B., Doherty, P.C., Gerhard, W., and Bennink, J., J. exp. Med. *145* (1977) 557.
77 Braciale, T.J., Cell. Immun. *33* (1977) 423.
78 Doherty, P.C., Effros, R.B., and Bennink, J., Proc. natn. Acad. Sci. USA *74* (1977) 1209.
79 Ennis, F.A., Martin, W.J., Verbonitz, M.W., and Butchko, G.M., Proc. natn. Acad. Sci. USA *74* (1977) 3006.
80 McMichael, A.J., Ting, A., Zweerink, H.J., and Askonas, B.A., Nature *270* (1977) 524.
81 Shaw, S., and Biddison, W.E., J. exp. Med. *149* (1979) 565.
82 Marshak, A., Doherty, P.C., and Wilson, D.B., J. exp. Med. *146* (1977) 1773.
83 McMichael, A.J., Gotch, F.M., Nable, G.R., and Beare, P.A.S., New Engl. J. Med. *309* (1983) 13.
84 Owen, J.A., Allouche, M., and Doherty, P.C., Cell. Immun. *67* (1982) 49.
85 Askonas, B.A., Müllbacher, A., and Ashman, R.B., Immunology *45* (1982) 79.
86 Townsend, A.R.M., Skehel, J.J., Taylor, P.M., and Palese, P., Virology *133* (1984) 456.
87 Townsend, A.R.M., McMichael, A.J., Carter, N.P., Huddleston, J.A., and Brownlee, G.G., Cell *39* (1984) 13.
88 Yewdell, J.W., Bennink, J.R., Smith, G.L., and Moss, B., Proc. natn. Acad. Sci. USA *82* (1985) 1785.
89 Yewdell, J.N., Frank, E., and Gerhard, W., J. Immun. *126* (1981) 1814.
90 Finberg, R., Spriggs, D.R., and Fields, B.N., J. Immun. *129* (1982) 2235.
91 Lamb, R.A., Zebedee, S.L., and Richardson, C.D., Cell *40* (1985) 627.
92 Lamb, R.A., and Choppin, P.W., Virologoy *112* (1981) 729.
93 Braciale, T.J., Braciale, V.L., Henkert, T.J., Sambrook, J., and Gething, M.J., J. exp. Med. *159* (1984) 341.
94 Bennink, J.R., Yewdell, J.W., Smith, G.L., Möller, C., and Moss, B., Nature *311* (1984) 578.
95 Bennink, J.R., Yewdell, J.W., and Gerhard, W., Nature *276* (1982) 75.
96 Doherty, P.C., Biddison, W.E., Bennink, J.R., and Knowles, B.B., J. exp. Med. *148* (1978) 534.
97 Zinkernagel, R.M., Althage, A., Cooper, S., Kreeb, G., Klein, P.A., Sefton, B., Flaherty, L.A., Stimpfling, J. Shreffler, D., and Klein, J., J. exp. Med. *148* (1978) 592.
98 Allouche, M., Owen, J.A., and Doherty, P.C., J. Immun. *129* (1982) 689.
99 Doherty, P.C., in: Proceedings of the Nobel Symposium on Genetics of the Immune Response, p.91. Eds G. and E. Möller. Plenum Press, New York 1983.
100 Müllbacher, A., Blanden, R.V., and Brenan, M., J. exp. Med. *157* (1983) 1324.
101 Klein, J., and Nagy, Z.A., Adv. Cancer Res. *37* (1982) 233.
102 Hodes, R.J., Germain, R.N., and Bluestone, J.A., Immun. today *5* (1984) 279.
103 Pan, S., and Knowles, B.B., Virology *125* (1983) 1.
104 Tevethia, S.S., Lewis, A.J., Campbell, A.E., Tevethia, M.J., and Rigby, P.W.J., Virology *133* (1984) 443.
105 O'Connell, K.., and Gooding, L.R., J. Immun. *132* (1984) 953.
106 McMichael, A., in: Options for the Control of Influenza. Eds A.P. Kendal and P.A. Patriarca. Alan R. Liss Inc. New York, N.Y. in press (1985).
107 Rock, K.L., and Benacerraf, B., J. exp. Med. *160* (1984) 1864.
108 Allan, J.E., and Doherty, P.C., Immunogenetics *21* (1985).581.

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Cell-extracellular matrix interactions in morphogenesis: an in vitro approach

by R. Montesano

Institute of Histology and Embryology, University of Geneva, Medical School, CH-1211 Geneva 4 (Switzerland)

Summary. We briefly review evidence from in vitro models that supports a role for the extracellular matrix in two essential steps of organogenesis: the establishment of appropriate three-dimensional cell-to-cell relationships, and the determination of a correct cell polarity.
Key words. Cell culture; organogenesis; collagen; endocrine pancreas; cell polarity; LLC-PK cells; endothelial cells; angiogenesis.

Formation of a precisely organized functional tissue or organ (organogenesis) is one of the major phases of embryonic development, and represents the culmination of a series of specific cellular events. Throughout the various stages of organogenesis, macromolecules present in the extracellular matrix provide structural support, and act as a glue that binds together the different cell types in a complex tissue or organ. Since the pioneering studies of