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COMMENTARY

Tissue Remodelling in the Adrenal Gland

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ABSTRACT. Adaptation of the adrenal gland to the demands of the organism is regulated functionally and structurally. Three common hypotheses on zonation in the adrenal gland, the migrational, zonal, and transformation field theories, try independently to reconcile the findings on structure, proliferation, and cell death. The classical theories on zonation are revisited in the light of recent data on cell death and renewal. In accordance with data on cell death as immunoreactivity against FAS (CD 95), an apoptosis-inducing receptor, *in situ* end labelling of fragmented DNA, and ultrastructural analyses, programmed cell death (PCD) occurs throughout the whole organ. The angiotensin II receptor subtypes described in the adrenal allow an additional regulation of tissue homeostasis by proliferative and even by the antiproliferative effects of the angiotensin II type 2 receptor. Proto-oncogenes are involved in the regulation of cell cycle and PCD, and adrenocorticotropin asserts its tissue integrating and differentiating effects by regulating proto-oncogenes such as c-*jun*, c-*fos*, *jun-B* and c-*myc*. Polypeptides involved in proliferation and DNA repair, such as proliferating cell nuclear antigen and Ki-67, have been found within zones of expected cell senescence. The expression of the class II major histocompatibility complex on normal adrenocortical cells allows cell-to-cell communication with the immune system and may trigger the Fas/Fas-ligand system to permit tissue regression and decreasing activity in both systems. In summary, new data allow us to reappraise and to reconcile the classical theories. Apoptosis is a physiological process in the adrenal gland. There is a differential regulation of apoptosis in the different zones. An investigation of this process may elucidate the basic mechanisms of adrenal zonation. BIOCHEM PHARMACOL **56**;2:163–171, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. adrenal gland; apoptosis; interaction; zonation

The adrenal gland, as a stress-responding organ, undergoes dynamic structural changes under physiological and pathophysiological conditions [1]. Those dynamic changes are covered by cell proliferation as well as cell death. Hormonal, paracrine, and intracellular signals initiate the processes required for these phenomena. Obviously, the regulated balance of both cell proliferation and cell death is a precondition for the appropriate functioning and integrity of the organ.

Regulatory systems of the organism are intermingled with local para- and autocrine systems as their intra-adrenal equivalent and predestine the stipulated balance of the cell cycle [2, 3]. These systems are explained not only by general mechanisms but also by their molecular and pharmacological properties. These properties are characterized by different receptor subtypes, new established agonists and antagonists, or the regulative mechanisms of the involved and related genes. The assessment of new metabolites and their receptors, such as the antiproliferative type 2 AII§ receptor, is possible by evaluating cell specific function, cell cycle, and cell death. In this context, the common theories of zonation in the adrenal gland need to be reevaluated.

Early works on the adrenal structure described the well-known histological division of the adrenal cortex into three different zones in their functional context. Attempts were made to answer questions concerning development and maintenance of the structure by investigating sites of cell proliferation and cell death. A frequent occurrence of dying cells exhibiting morphological signs different from necrosis was found in the zona reticularis [4]. In general, those morphological apoptotic signs occur in single cells and consist of chromatin condensation, shrinkage of the nucleus, condensation of the cytoplasm, convolution and protrusion of nuclear and plasma membrane, and formation of membrane-engulfed remnants containing well-preserved cyto-organelles [5]. The signs distinguishing necrosis from programmed cell death are, among others, the loss of

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§ *Abbreviations:* ACTH, adrenocorticotropin; AII, angiotensin II; AT2, angiotensin II type 2 receptor; CRH, corticotropin releasing hormone; DHEA, dehydroepiandrostenedione; HPA-axis, hypothalamus–pituitary–adrenal axis; ISEL, *in situ* end labelling; MHC, major histocompatibility complex; PCNA, proliferating cell nuclear antigen; and TNF-α, tumor necrosis factor-α.

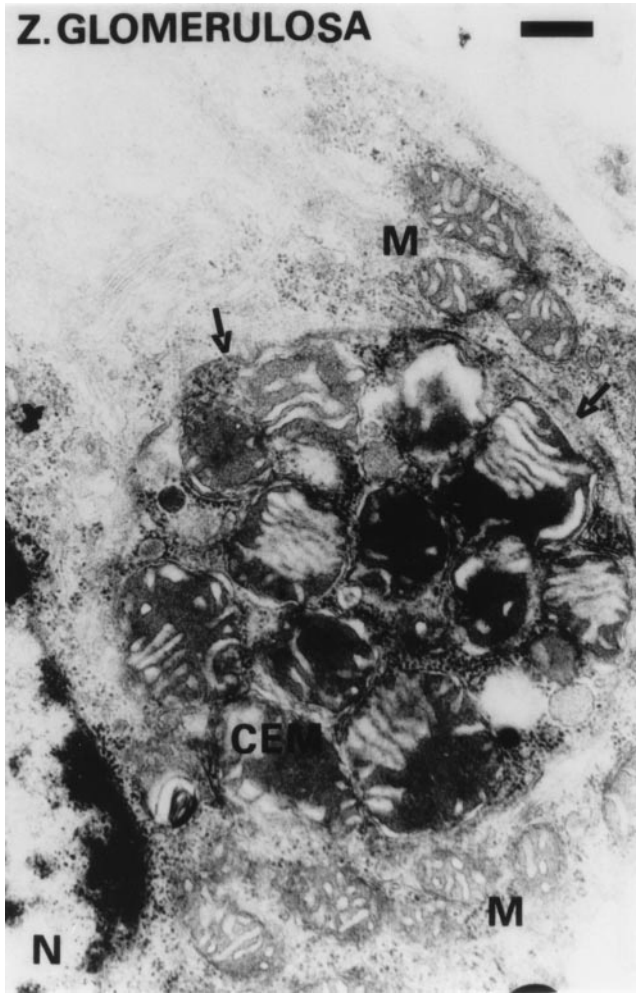

FIG. 1. Electron micrograph of human adrenal cortex. A membrane-engulfed apoptotic body (arrows) is shown with characteristic mitochondria (CEM) of epithelial origin. The phagocytoting cell belongs to the zona glomerulosa as indicated by mitochondria (M), provided with sickle-forming tubuli. The nucleus of the phagocytoting cell (N) is not affected by apoptosis (bar = 0.1 μm). Reprinted with permission from *J Clin Endocrinol Metab* 81: 4129–4136, 1996. © 1996 The Endocrine Society. [Ref. 27].

membrane integrity, cell swelling and lysis, lysosomal leakage, ill-defined chromatin aggregation, and inflammatory response [6]. The investigation of adrenal tissue with histological and ultrastructural techniques led to the identification of the process now known as apoptosis [5, 7–10] (see Fig. 1). In this commentary, the classical theories of adrenal zonation are revisited, and an attempt is made at a new interpretation in light of recent data on the regulation of apoptosis and cell proliferation.

ADRENAL ZONATION—THREE CLASSICAL THEORIES

Varying findings on cell proliferation and cell death led to at least three different hypotheses on zonation in the adrenal gland [1].

The **migration theory** (Fig. 2, A1) describes the proliferation of cortical cells in the outermost layers of the adrenal cortex and the growth of new cells towards the medulla [11–13]. These findings have been supported by the autoradiographic detection of S-phase cells within the zona glomerulosa and the outer zona fasciculata. The highest mitotic activity could be demonstrated within a region located between the zona glomerulosa and the zona fasciculata. Therefore, an intermediate zone of proliferating cells has been introduced [14–16] (Fig. 2, A2). Belloni *et al.* [17] suggested loop-forming cords from the intermediate zone into and passing the zona glomerulosa with a final migration towards the medulla (Fig. 2, A1). On the other hand, the dying cells had been found mainly in the inner parts of the adrenal cortex, so the zona reticularis was seen as a zone of cell senescence [4, 8, 9]. Functional investigations with ACTH deprivation [10] and toxic or hypoxic stress [4] resulted in increased understanding of the high cell turnover of the adrenal gland. The ultrastructural analysis carried out by Kerr, Wyllie and coworkers also strongly supported this theory; here, morphologic signs of apoptosis were examined under different activation conditions mainly within the zona reticularis [9].

Another theory, the **transformation field theory**, suggests two transformational directions (Fig. 2B). The *progressive* transformation involves the replacement of zonal tissue by fasciculata cells under physiological activation; the *regressive* transformation results in the disappearance of these fasciculata cells with the glomerulosa and reticularis cells remaining. The misconception was the assumption of two remodeling and transformational fields [18–20]. These transformational fields were thought to be located between the zona glomerulosa and the zona fasciculata and between the zona fasciculata and the zona reticularis. The proliferating capacity would then have been dependent on the fasciculata zone and its regulatory mechanisms.

A third **zonal theory** involves the equal proliferation of all three zones [21–24] based on the observation that mitoses of [^{3}H]thymidine-labeled S-phase cells occur in all three zones, including the zona reticularis. Each zone could then be regulated on its own without affecting any other zone in its functional behavior (see Fig. 2C).

All of these theories attempt to answer the phenomenon of maintenance of structural homeostasis as a relationship between cell proliferation and cell death in the adrenal gland.

INTERFERENCE OF NEW DATA ON CELL DEATH AND RENEWAL AND INTRA-ADRENAL CELLULAR COMMUNICATION WITH THE CLASSICAL THEORIES

Cell Death and Its Mediators

The classical theories of adrenal zone formation need to be reevaluated in the light of new data on apoptosis obtained by modern techniques, such as ISEL of fragmented DNA [25]. The staining of human adrenal glands by ISEL shows

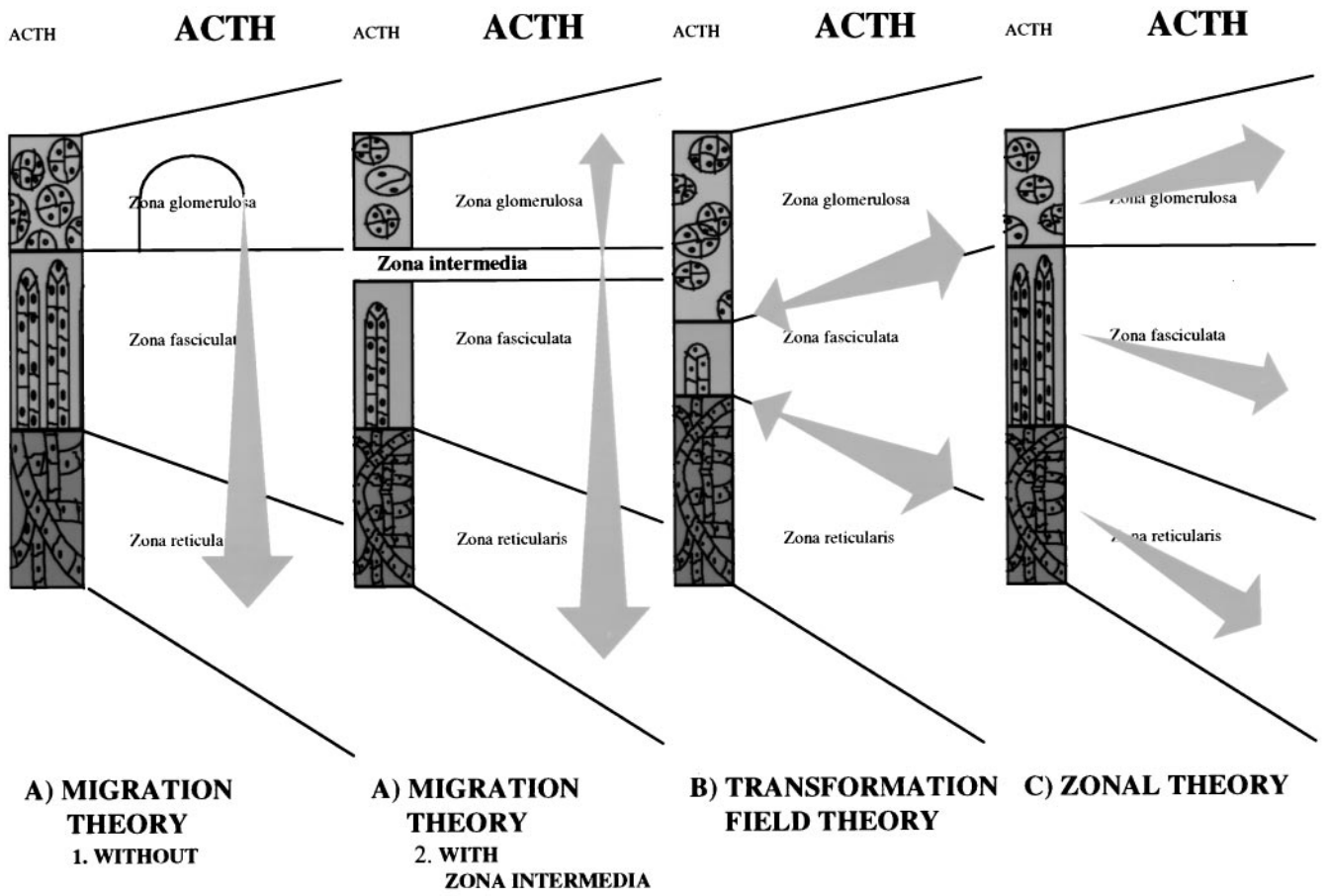

FIG. 2. Classical theories of adrenal zone formation. The origin of the arrows marks the place of cell renewal and proliferation, whereas the tips indicate the directions of growth and/or migration after stimulation (ACTH). (A) Migration theory. Mitoses and signs of proliferation have been found mainly in the zona glomerulosa and the outer zona fasciculata. (1) Cords run straight from the zona glomerulosa towards the medulla or form loops starting between the zona glomerulosa and the outer zona fasciculata. (2) Bidirectional migration starting from an intermedia zone. (B) Transformation field theory. Two transformational fields are suggested to be located between the zona glomerulosa and the zona fasciculata and the zona fasciculata and the zona reticularis, respectively. *Progressive* transformation results in a proliferative increase of the fasciculata zone; E*regressive* transformation leads to a narrowing and functional restriction. (C) Zonal theory. Independent proliferation of all three zones has been suggested, as a low number of mitoses have been proven in all adrenocortical zones.

positive signals throughout the whole organ, albeit with a differing appearance in all three zones. The staining intensity in the medulla is comparable to the inner cortical zones. The morphometric analysis of the apoptotic rate in the three zones of the adrenal cortex varies among different investigators, depending on the procedure followed. In general, the labelling of fragmented DNA is possible in apoptotic, necrotic, and S-phase cells. Therefore, an additive effect of apoptotic and at least S-phase cells is assumed. A positive labelling in the zona glomerulosa and the zona reticularis is consistent [26, 27] (Fig. 3). Furthermore, the AII receptor distribution in the adrenal gland has been described [28], and the existence of the antiproliferative AT 2 receptors has been shown within the zona glomerulosa [29].

CD 95 (also called FAS or APO-1), a cell surface antigen, is known to be a polypeptide involved in the induction of apoptosis by its cross-linking [30, 31]. Leithäuser *et al.* [32] showed CD 95-like immunoreactivity in all zones of the adrenal cortex.

Immunoreactivity against p53, activated by DNA damage and promoting G_1 phase arrest or apoptosis, was shown in correlation with p21, its downstream effector, blocking progression into the S-phase and allowing time for DNA repair. This immunoreactivity is found mainly in the inner cortical layers [33] and occurs due to ischemic injury. The role of p53 in the adrenal structure and function has been studied in adrenal neoplasms, where mutations result in a loss of function and subsequent neoplastic degeneration [34, 35].

Proliferation and Cell Renewal

The data on functional regulation and stimulation of the zona glomerulosa reveal a local renin-angiotensin system [36], the stimulating effects of arginine-vasopressin [37, 38],

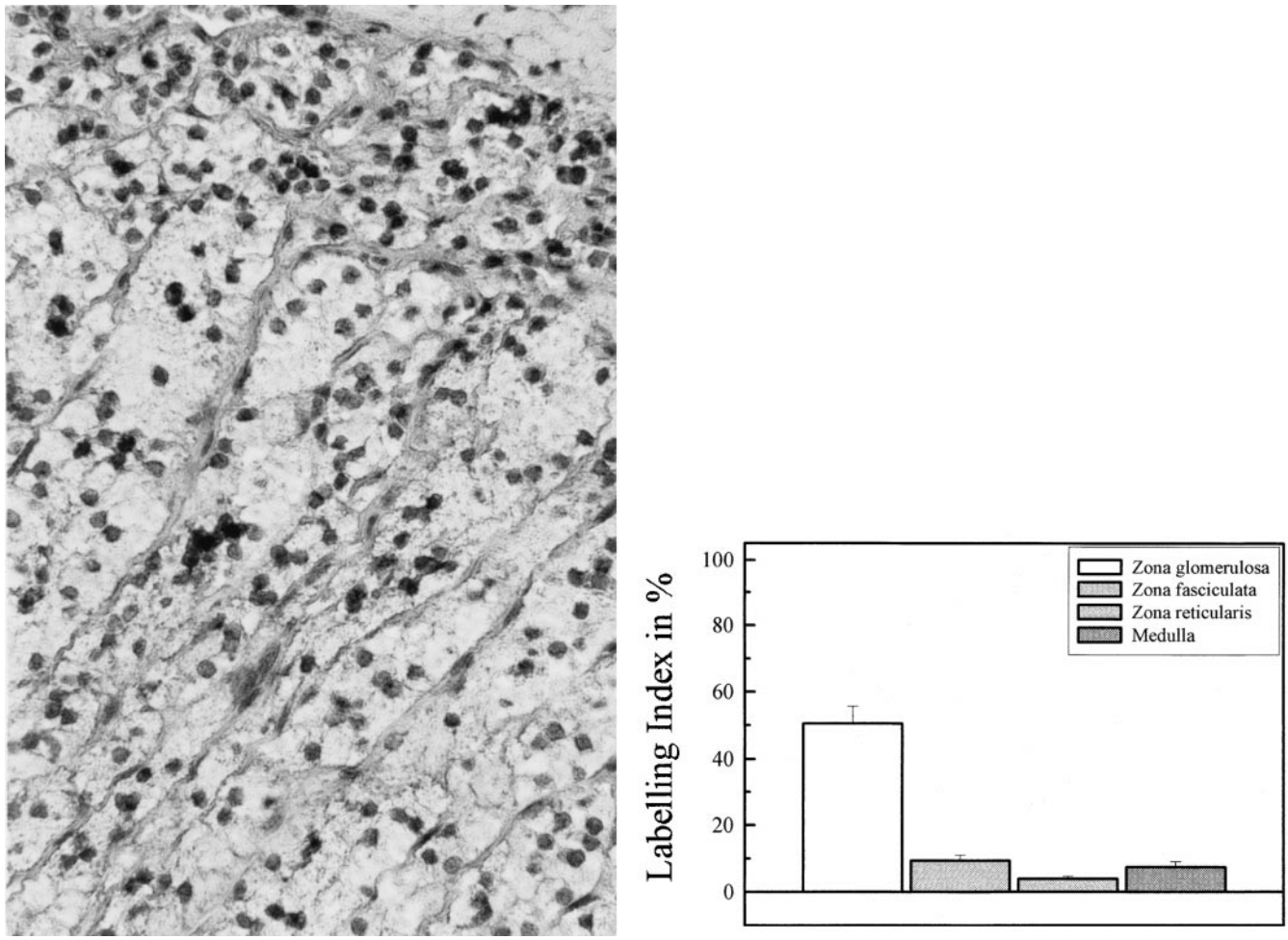

FIG. 3. Left panel: Cryosection of a normal adrenal gland stained by ISEL of fragmented DNA with digoxigenin-marked deoxy-UTP and subsequent immunostaining. A positive brown color product within the nuclei is detectable in all zones of the adrenal cortex (bar = 50 μm). Right panel: Apoptosis in normal human adrenal. The graph shows the labelling index (LI) for each zone of the adrenal cortex and for the medulla obtained by ISEL. The highest index is notable in the zona glomerulosa and decreases toward the medulla. Values are means ± SEM, N = 10. LI_{glom} = 50.46 ± 5.22%; LI_{fasc} = 9.36 ± 1.68%; LI_{ret} = 3.90 ± 0.78%; and LI_{med} = 7.37 ± 1.62%. The right panel of this figure is reprinted with permission from *J Clin Endocrinol Metab* 81: 4129–4136, 1996. © 1996 The Endocrine Society. [Ref. 27].

and the need of vitamins and anti-oxidants in regular functioning [39]. The proliferation-stimulating effect of ACTH has been indicated by the rise of basic fibroblast growth factor and insulin-like growth factor-I mRNAs within the glomerulosa zone [40].

Immunostaining to Ki-67, an antigen associated with cell proliferation [41], was shown by Sasano *et al.* [26] in human adrenals predominantly in the outer fasciculata but also in the glomerulosa and reticularis.

The stimulatory effect of ACTH on tissue-integrating and differentiation-regulating proto-oncogenes such as c-*jun* and *jun-B* [42], and c-*myc* [43] has been investigated in rats and humans. An increase in c-fos and jun-B mRNA levels after ACTH exposure and an increase of c-jun, c-fos and jun-B after AII stimulation has been reported in bovine and ovine fasciculata cells [44]. Transcription factors also were implicated in the regulation of the cell cycle and programmed cell death [45, 46].

Immunoreactivity against PCNA is found mainly in the zona reticularis [27]. PCNA is defined as a polypeptide whose synthesis reaches a peak during the S-phase of the cell cycle. It is also expressed during DNA repair and, therefore, has been identified as the polymerase delta subunit [47–49].

Intra-adrenal Regulation and Interaction

Recently, the communication between adrenocortical cells via gap junctions was shown to take part in intra-adrenal regulation [50]. Gap junctions have been detected mainly within the zona fasciculata and the zona reticularis [51], and apoptosis is known to occur in dispersed and separated cells [52]. In recent years, it has been well established that neuropeptides, neurotransmitters, growth factors, and cytokines participate in intra-adrenal regulation (for review, see Ref. 3). Chromaffin cells can be seen throughout the entire adrenal cortex, and cortical cells are found frequently

within the medulla. The two cell types exhibit close cellular connections as indicated by ultrastructural analysis [52]. Therefore, catecholamines and other neuropeptides produced by chromaffin cells can directly regulate steroidogenesis in all zones of the cortex [2, 53, 54].

Immuno-adrenal Interaction

There is an interaction between the immune system and the HPA-axis [55–57]. Immune cells such as macrophages, mast cells, and lymphocytes are to be found in the adrenal gland. These cells have been shown to interact with adrenocortical cells [58]. Macrophages in the adrenal gland belong to the phagocytotic compartment as characterized by the expression of Ki-M8 and the adhesion molecule CD 11 and CD 68 and could serve as scavengers removing the apoptotic bodies and remnants in this system. Macrophages are known to be producers and/or effectors of cytokines and are considered as secretory cells of the immune system [59]. Hence, they could share an immunological function with MHC class II-expressing cells of the inner cortical layers of the adrenal gland. The expression of the MHC class II (i.e. HLA-DR) on antigen-presenting cells and on some epithelial cells is well described [60]. Nevertheless, weak MHC class II immunostaining was found in the inner zona fasciculata, whereas strong staining occurred in the zona reticularis [61, 62]. A functional relationship to the immune system by the expression of MHC class II is evident. Interestingly, MHC class II expression occurs only in normal and benign neoplastic human adrenal tissues, whereas adrenocortical carcinomas lack this expression [63, 64]. On the contrary, MHC class II expression is high in the functionally insufficient adrenal glands in Addison's disease [56].

Evidence is mounting for paracrine and/or autocrine regulation of cell death. Interleukin-1 is regarded as an inhibitor of apoptosis [65], and its expression and distribution in the adrenal cortex could be shown with high prevalence in the inner zones of the cortex [66]. The mitogenic factor interleukin-6 has also been detected in the inner zones [67]. Other cytokines also known to be positive mediators of apoptosis, such as TNF-α, have been found in the inner cortical layers of the adrenal gland, and 17α-hydroxylase cytochrome P450-positive cells co-express TNF-α mRNA and MHC class II, as detected by *in situ* hybridization and double-immunostaining [32, 68]. Interestingly, TNF-α is also an important mediator of MHC class II expression [69, 70].

REAPPRAISAL OF ADRENAL ZONATION IN THE LIGHT OF APOPTOSIS AND CELL PROLIFERATION

It is possible to imagine programmed cell death as a coregulated factor of tissue integrity as well as of functional demands at the place of replication and proliferation, during functional differentiation, migration, or near the structural borders of the organ. The occurrence of apoptosis in all three adrenocortical zones with their specific products could guarantee a regulation at any stage of a cell's development. As each of the three zones produces its own specific hormones, another means to adapt to the demands of the organism might be given.

Hence, zonal selection or prevalence for programmed cell death needs special regulatory mechanisms, which would be reflected by differing apoptotic indices, associated protein expression, and methods of scavenging for apoptotic remnants. Classical ways of inducing the process of programmed cell death in the adrenal gland are evident, e.g. the control of tissue proliferation and homeostasis by trophic hormones, the blunting of hyperactive and hyperproliferative cells, the control of damaged DNA, cell injury, and the protection of the cell cycle, and, finally, the simple senescence of cells lacking sufficient telomerase activity [71].

Data agree with the observations leading to the concept of the **migration theory**. The adrenal cortex is controlled by the trophic hormone ACTH, which up-regulates proliferation in respect to the functional demands of the entire organism. The stimulation of the cortex results in the proliferation of the zona fasciculata [37]. Obviously, effective mechanisms protecting from over-activation or neoplastic degeneration after dysregulated proliferation are necessary. The withdrawal of ACTH in primary cultures leads to apoptosis in cells positive in 17α-hydroxylase immunostaining [72]. Interestingly, the remaining cells were negative for 17α-hydroxylase but positive for PCNA immunostaining. A recently established model of the mouse steroid 21-hydroxylase/β-galactosidase transgene clearly shows the clonal expansion and migration of the cells, creating a fascicle-like aspect [73].

The **transformational field theory** is also of interest in light of the new data. CD 95 expression was found by some investigators in all three zones of the cortex. In rats, an increase of the zona glomerulosa is described, while the zona intermedia decreases during administration of AII and a low salt diet [74]. The existence of the zona intermedia itself [75, 76] supports the theory of "transformation" towards glomerulosa or fasciculata or "migration" in both directions, at least in rodents.

Finally, aspects of the **zonal theory** agree with findings of basic cell growth and cell molting rates during ACTH withdrawal, which exist either depending on stimuli such as CRH [77] or of the adrenal medulla, or additional external stimulation. This might be the case in Addison's disease or in the phenomenon of irreversible cortex suppression due to clinical ACTH deprivation during long-term glucocorticoid application and could create the aspect of very thin fasciculata and reticularis zones. The cell death rate may then be a result of very low telomerase activity, reflecting quiet senescence. Cell death triggered by DNA damage via injuries, as reported by Didenko *et al.* [33], is not related to

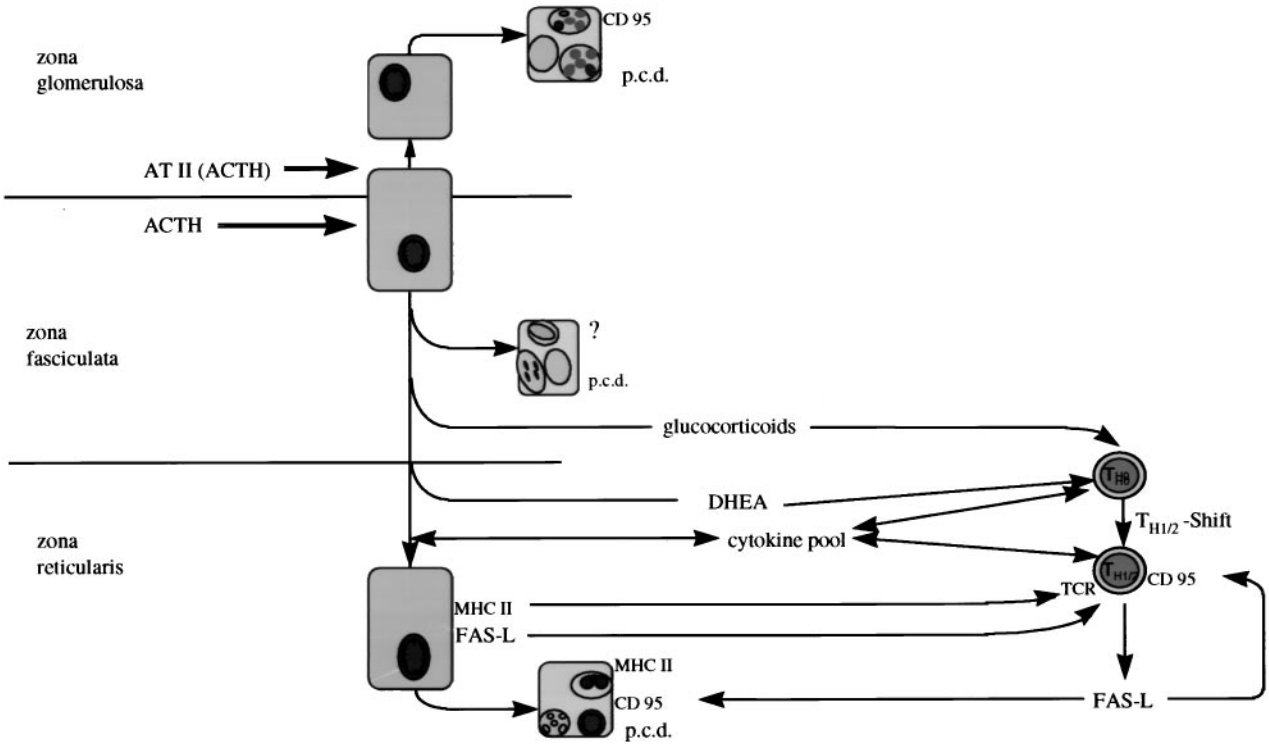

FIG. 4. Differential regulation of apoptosis and zonation in the adrenal gland. Apoptosis, beside other mechanisms, guarantees a zone-related regulation, depending on the needs for zone-specific products at the sites of proliferation, growth, migration, and differentiation. Apoptosis is detectable in all cortical layers [sites of programmed cell death (p.c.d.)]. Likewise, receptors, known because of their antiproliferative and apoptosis-inducing effects such as CD 95 and AT 2, have been found in the adrenal cortex. Zone-specific products, such as glucocorticoids and DHEA, assert modulation effects on Th0 cells and trigger a function shift towards Th1 or Th2. High differentiation of cortical cells and the cytokine pool promote MHC class II (MHC II) expression. MHC class II interacts with the T-cell receptor (TCR) and triggers FAS-ligand (FAS-L) expression. FAS-L induces apoptosis in both adrenocortical cells and T-cells.

any special zone, but it should refer to the afflicted zone. In addition, the distribution of proliferation markers could not be clearly localized to any special zone. A recent study shows an independent regulation of DNA synthesis in all three zones [75]. The distribution of CD 95 [27, 30] and also the occurrence of fragmented, nucleolar DNA [27, 78] and ultra-structural investigations [27, 34] enable the definition of apoptosis in all adrenal zones.

There is evidence for CD 95 action and internucleosomal DNA fragmentation in outer cortical layers. Only a few macrophages, and even fewer cytokines, have been detected in these parts of the cortex. Hence, epithelial cells have to scavenge the remnants themselves. This agrees with ultrastructural analyses [27]. The signaling and modulation of programmed cell death through known regulators of the zona glomerulosa, such as AII, have not been established yet. Nevertheless, the expression of the appropriate AT 2 receptors within the zona glomerulosa is evident, and its involvement in the process of apoptosis has been suggested, although it has not yet been proven for the adrenal gland [79, 80].

Inner cortical layers have been reported to show apoptotic signs such as cellular shrinkage and the formation of apoptotic bodies. Hence, it is exciting to speculate as to whether a regulated circuit of inner cortical cells switching on the expression of MHC class II on the one hand and scavenging macrophages on the other could exist, which might well be regulated by a pool of cytokines and interplay with the natural MHC II-corresponding cells, the T-lymphocytes. A subtype shift of T-lymphocytes towards Th1 function in the milieu of high DHEA concentration is possible, whereas glucocorticoids promote a subtype shift towards Th2 function [81–84]. The interaction with MHC could initiate the expression of the FAS-ligand in both adrenocortical cells and lymphocytes and trigger the programmed cell death in cortical cells as well as in T-lymphocytes themselves [85] (see Fig. 4). The latter phenomenon is known as *activation-induced apoptosis* of the T-lymphocytes [86]. It has already been shown that apoptosis in lymphocytes is mediated via MHC class II [87], and that MHC class II enhances sensitivity to CD 95-mediated apoptosis [88]. This could serve to avoid autoimmune stressors and regulate homeostatic immune response [89, 90]. A mechanism of programmed cell death mediated via MHC class II has been reported in B-lymphocytes [91]. Furthermore, even soluble MHC class II molecules induce apoptosis in T-lymphocytes [92].

CONCLUSION AND PERSPECTIVES

The apoptotic markers provide fascinating inroads for analyzing adrenal function under stress and during pathophysiological stages. The apoptosis has an integrating effect on local tissue structure and protects from neoplastic transformation. There is a differential regulation of apoptosis in the different zones. In addition, the assessment of apoptotic parameters, together with MHC class II detection, allows differentiation between benign and malignant neoplasms in the case of adrenal masses discovered incidentally [63, 64], when common histological examinations [93, 94] fail to give a clear assessment. The MHC class II molecules indicate a local, intra-adrenal connection between the HPA-axis and the immune system. Besides the local effect, there may be a function in T-cell selection to promote anergy and protect from autoimmunity. A repercussion on the process of ageing might be possible, as MHC class II expression correlates with DHEA production and declines over a lifetime [95, 96]. It will be exciting to further elucidate these functional aspects of adrenal cell physiology under both normal and pathological conditions.

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References

1. Nussdorfer GG, Cytophysiology of the adrenal cortex. *Int Rev Cytol* **98:** 1–405, 1986.
2. Bornstein SR, Ehrhart-Bornstein M and Scherbaum WA, Morphological and functional studies of the paracrine interaction between cortex and medulla in the adrenal gland. *Microsc Res Tech* **36:** 520–533, 1997.
3. Ehrhart-Bornstein M, Bornstein SR and Scherbaum WA, Sympathoadrenal system and immune system in the regulation of adrenocortical function. *Eur J Endocrinol* **135:** 19–26, 1996.
4. Hoerr NL, The cells of the suprarenal cortex in the guinea-pig. *Am J Anat* **48:** 139–197, 1931.
5. Kerr JF, Wyllie AH and Currie AR, Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* **26:** 239–257, 1972.
6. Thompson HJ, Strange R and Schedin PJ, Apoptosis in the genesis and prevention of cancer. *Cancer Epidemiol Biomarkers Prev* **1:** 597–602, 1992.
7. Idelman S, Ultrastructure of the mammalian adrenal cortex. *Int Rev Cytol* **27:** 181–281, 1970.
8. Kerr JF, Shrinkage necrosis of adrenal cortical cells. *J Pathol* **107:** 217–219, 1972.
9. Wyllie AH, Kerr JF and Currie AR, Cell death in the normal neonatal rat adrenal cortex. *J Pathol* **111:** 255–261, 1973.
10. Wyllie AH, Kerr JF, Macaskill IA and Currie AR, Adrenocortical cell deletion: The role of ACTH. *J Pathol* **111:** 85–94, 1973.
11. Gottschau M, Structur und embryonale Entwicklung der Nebenniere bei Säugetieren. *Arch Anat Physiol* **8:** 412–457, 1883.
12. da Costa C, Histophysiologie du cortex surrenal. *Ann Endocrinol (Paris)* **12:** 361–403, 1951.
13. Idelman S, General comparative and clinical endocrinology of adrenal cortex. In: *Adrenal Cortex* (Ed. Jones IC), pp. 1–199. Academic Press, London, 1978.
14. Sugihara H, Kawai K and Tsuchiyama H, Effects of exogenous ACTH on the rat fetal adrenal cortex: A histochemical and electron-microscopical observation. *Acta Pathol Jpn* **27:** 477–484, 1977.
15. Orenberg EK and Glick D, Quantitative histologic distribution of adenosine-3′,5′-monophosphate in the rat adrenal and effect of adrenocorticotropin. *J Histochem Cytochem* **20:** 923–928, 1972.
16. Golder MP and Boyns AR, Distribution of adrenocorticotrophic hormone-stimulated adenylate cyclase in the adrenal cortex. *J Endocrinol* **56:** 471–481, 1973.
17. Belloni AS, Mazzocchi G, Meneghelli V and Nussdorfer GG, Cytogenesis in the rat adrenal cortex: Evidence for an ACTH-induced centripetal cell migration from the zona glomerulosa. *Arch Anat Histol Embryol* **61:** 195–205, 1978.
18. Yoffey JM, The suprarenal cortex: The structural background. In: *The Suprarenal Cortex* (Ed. Yoffey JM), pp. 31–38. Academic Press, New York, 1953.
19. Tonutti E, Über die strukturelle Funktionsanpassung der Nebennierenrinde. *Endokrinologie* **28:** 1–15, 1951.
20. Chester-Jones I, *The Adrenal Cortex*. Cambridge University Press, London, 1957.
21. Swann HG, The pituitary-adrenocortical relationship. *Physiol Rev* **20:** 493–521, 1940.
22. Sarason EL, Morphological changes in the rat's adrenal cortex under various experimental conditions. *Arch Pathol* **35:** 373–390, 1997.
23. Malendowicz LK, Robba C and Nussdorfer GG, Sex differences in adrenocortical structure and function. XXII. Light- and electron-microscopic morphometric studies on the effects of gonadectomy and gonadal hormone replacement on the rat adrenal cortex. *Cell Tissue Res* **244:** 141–145, 1986.
24. Nag B, Arimilli S, Mukku PV and Astafieva I, Functionally active recombinant α and β chain-peptide complexes of human major histocompatibility class II molecules. *J Biol Chem* **271:** 10413–10418, 1996.
25. Compton MM, A biochemical hallmark of apoptosis: Internucleosomal degradation of the genome. *Cancer Metastasis Rev* **11:** 105–119, 1992.
26. Sasano H, Imatani A, Shizawa S, Suzuki T and Nagura H, Cell proliferation and apoptosis in normal and pathologic human adrenal. *Mod Pathol* **8:** 11–17, 1995.
27. Wolkersdorfer GW, Ehrhart-Bornstein M, Brauer S, Marx C, Scherbaum WA and Bornstein SR, Differential regulation of apoptosis in the normal human adrenal gland. *J Clin Endocrinol Metab* **81:** 4129–4136, 1996.
28. Clauser E, Curnow KM, Davies E, Conchon S, Teutsch B, Vianello B, Monnot C and Corvol P, Angiotensin II receptors: Protein and gene structures, expression and potential pathological involvements. *Eur J Endocrinol* **134:** 403–411, 1996.
29. Shanmugam S, Llorens-Cortes C, Clauser E, Corvol P and Gasc J-M, Expression of angiotensin II AT_2 receptor mRNA during development of rat kidney and adrenal gland. *Am J Physiol* **268:** F922–F930, 1995.
30. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y and Nagata S, The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* **66:** 233–243, 1991.
31. Trauth BC, Klas C, Peters AM, Matzku S, Moller P, Falk W, Debatin KM and Krammer PH, Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* **245:** 301–305, 1989.
32. Leithäuser F, Dhein J, Mechtersheimer G, Koretz K, Brüderlein S, Henne C, Schmidt A, Debatin K-M, Krammer PH and

Möller P, Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. *Lab Invest* **69:** 415–429, 1993.
33. Didenko VV, Wang X, Yang L and Hornsby PJ, Expression of $p21^{(WAF1/CIP1/SDI1)}$ and p53 in apoptotic cells in the adrenal cortex and induction by ischemia/reperfusion injury. *J Clin Invest* **97:** 1723–1731, 1996.
34. Reincke M, Karl M, Travis WH, Mastorakos G, Allolio B, Linehan HM and Chrousos GP, p53 mutations in human adrenocortical neoplasms: Immunohistochemical and molecular studies. *J Clin Endocrinol Metab* **78:** 790–794, 1994.
35. Lin S-R, Lee Y-J and Tsai J-H, Mutations of the p53 gene in human functional adrenal neoplasms. *J Clin Endocrinol Metab* **78:** 483–491, 1994.
36. Gupta P, Franco-Saenz R and Mulrow PJ, Locally generated angiotensin II in the adrenal gland regulates basal, corticotropin-, and potassium-stimulated aldosterone secretion. *Hypertension* **25:** 443–448, 1995.
37. Bähr V, Sander-Bähr C, Hensen J and Oelkers W, Influence of sodium and potassium diets on adrenal vasopressin content and direct effects of vasopressin on aldosterone synthesis in adrenocortical cells. *Horm Metab Res* **25:** 411–416, 1993.
38. Shanmugam S, Monnot C, Corvol P and Gasc J-M, Distribution of type I angiotensin II receptor subtype messenger RNAs in the rat fetus. *Hypertension* **23:** 137–141, 1994.
39. Redmann A, Mobius K, Hiller HH, Oelkers W and Bahr V, Ascorbate depletion prevents aldosterone stimulation by sodium deficiency in the guinea pig. *Eur J Endocrinol* **133:** 499–506, 1995.
40. Ho MM and Vinson GP, Endocrine control of the distribution of basic fibroblast growth factor, insulin-like growth factor-I and transforming growth factor-β, mRNAs in adult rat adrenals using non-radioactive *in situ* hybridization. *J Endocrinol* **144:** 379–387, 1995.
41. Gerdes J, Lemke H, Baisch H, Wacker H-H, Schwab U and Stein H, Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* **133:** 1710–1715, 1984.
42. Le Houx JG and Ducharme L, *In vivo* effects of adrenocorticotropin on c-jun, jun-B, c-fos and fos-B in rat adrenal. *Endocr Res* **21:** 267–274, 1995.
43. Liu J, Voutilainen R, Kahri AI and Heikkilä P, Expression of the c-*myc* gene in human adrenals: Regulation by adrenocorticotropin in primary cultures. *J Endocrinol* **148:** 523–529, 1996.
44. Viard I, Hall SH, Jaillard C, Berthelon MC and Saez JM, Regulation of c-fos, c-jun and jun-B messenger ribonucleic acids by angiotensin-II and corticotropin in ovine and bovine adrenocortical cells. *Endocrinology* **130:** 1193–1200, 1992.
45. King KL and Cidlowski JA, Cell cycle and apoptosis: Common pathways to life and death. *J Cell Biochem* **58:** 175–180, 1995.
46. Buttyan R, Zakeri Z, Lockshin R and Wolgemuth D, Cascade induction of c-*fos*, c-*myc*, and heat shock 70K transcripts during regression of the rat ventral prostate gland. *Mol Endocrinol* **2:** 650–657, 1988.
47. Mathews MB, Bernstein RM, Franza BR Jr and Garrels JI, Identity of the proliferating cell nuclear antigen and cyclin. *Nature* **309:** 374–376, 1984.
48. Shivji KK, Kenny MK and Wood RD, Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* **69:** 367–374, 1992.
49. Waseem NH and Lane DP, Monoclonal antibody analysis of the proliferating cell nuclear antigen (PCNA). Structural conservation and the detection of a nucleolar form. *J Cell Sci* **96:** 121–129, 1990.
50. Munari-Silem Y, Lebrethon MC, Morand I, Rousset B and Saez JM, Gap junction-mediated cell-to-cell communication in bovine and human adrenal cells. A process whereby cells increase their responsiveness to physiological corticotropin concentrations. *J Clin Invest* **95:** 1429–1439, 1995.
51. Murray SA, Oyoyo UA, Pharrams SY, Kumar NM and Gilula NB, Characterization of gap junction expression in the adrenal gland. *Endocr Res* **21:** 221–229, 1995.
52. Bornstein SR, Ehrhart-Bornstein M and Scherbaum WA, Ultrastructural evidence for cortico-chromaffin hybrid cells in rat adrenals? *Endocrinology* **129:** 1113–1115, 1991.
53. Ehrhart-Bornstein M, Bornstein SR, Trzeclak WH, Usadel H, Guse-Behling H, Waterman MR and Scherbaum WA, Adrenaline stimulates cholesterol side-chain cleavage cytochrome P450 mRNA accumulation in bovine adrenocortical cells. *J Endocrinol* **131:** R5–R8, 1991.
54. Bornstein SR, González-Hernández JA, Ehrhart-Bornstein M, Adler G and Scherbaum WA, Intimate contact of chromaffin and cortical cells within the human adrenal gland forms the cellular basis for important intraadrenal interactions. *J Clin Endocrinol Metab* **78:** 225–232, 1994.
55. Chrousos GP, The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *N Engl J Med* **332:** 1351–1362, 1995.
56. Axelrod J and Reisine TD, Stress hormones: Their interaction and regulation. *Science* **224:** 452–459, 1984.
57. Besedovsky HO and del Rey A, Immune-neuro-endocrine interactions: Facts and hypotheses. *Endo Rev* **17:** 64–102, 1996.
58. González-Hernández JA, Bornstein SR, Ehrhart-Bornstein M, Geschwend JE, Adler G and Scherbaum WA, Macrophages within the human adrenal gland. *Cell Tissue Res* **278:** 201–205, 1994.
59. Nathan CF, Secretory products of macrophages. *J Clin Invest* **79:** 319–326, 1987.
60. Germain RN, MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation. *Cell* **76:** 287–299, 1994.
61. Khoury EL, Greenspan JS and Greenspan FS, Adrenocortical cells of the zona reticularis normally express HLA-DR antigenic determinants. *Am J Pathol* **127:** 580–591, 1987.
62. McNicol AM, Class II MHC antigen expression in adrenal cortex. *Lancet* **2:** 1282, 1986.
63. Marx C, Wolkersdörfer GW, Brown JW, Scherbaum WA and Bornstein SR, MHC class II expression—A new tool to assess dignity in adrenocortical tumours. *J Clin Endocrinol Metab* **81:** 4488–4491, 1996.
64. Wolkersdörfer GW, Marx C, Brown JW, Scherbaum WA and Bornstein SR, Evaluation of apoptotic parameters in normal and neoplastic human adrenal. *Endocr Res* **22:** 411–419, 1996.
65. McConkey DJ, Hartzell P, Chow SC, Orrenius S and Jondal M, Interleukin 1 inhibits T cell receptor-mediated apoptosis in immature thymocytes. *J Biol Chem* **265:** 3009–3011, 1990.
66. González-Hernández JA, Bornstein SR, Ehrhart-Bornstein M, Gschwend JE, Gwosdow A, Jirikowski G and Scherbaum WA, IL-1 is expressed in human adrenal gland *in vivo*. Possible role in a local immune-adrenal axis. *Clin Exp Immunol* **99:** 137–141, 1995.
67. González-Hernández JA, Bornstein SR, Ehrhart-Bornstein M, Spath-Schwalbe E, Jirikowski G and Scherbaum WA, Interleukin-6 messenger ribonucleic acid expression in human adrenal gland *in vivo*: New clue to a paracrine or autocrine regulation of adrenal function. *J Clin Endocrinol Metab* **79:** 1492–1497, 1994.
68. González-Hernández JA, Ehrhart-Bornstein M, Späth-Schwalbe E, Scherbaum WA and Bornstein SR, Human adrenal cells express tumor necrosis factor-α messenger ribo-

nucleic acid: Evidence for paracrine control of adrenal function. *J Clin Endocrinol Metab* **81:** 807–813, 1996.
69. Pfizenmaier K, Scheurich P, Schlüter C and Krönke M, Tumor necrosis factor enhances HLA-A,B,C and HLA-DR gene expression in human tumor cells. *J Immunol* **138:** 975–980, 1987.
70. Watanabe Y and Jacob CO, Regulation of MHC class II antigen expression. Opposing effects of tumor necrosis factor-α on IFN-γ-induced HLA-DR and Ia expression depends on the maturation and differentiation stage of the cell. *J Immunol* **146:** 899–905, 1991.
71. Harley CB, Futcher AB and Greider CW, Telomeres shorten during ageing of human fibroblasts. *Nature* **345:** 458–460, 1990.
72. Negoescu A, Labat-Moleur F, Defaye G, Mezin P, Drouet C, Brambilla E, Chambaz EM and Feige JJ, Contribution of apoptosis to the phenotypic changes of adrenocortical cells in primary culture. *Mol Cell Endocrinol* **110:** 175–184, 1995.
73. Morley SD, Viard I, Chung B-C, Ikeda Y, Parker KL and Mullins JJ, Variegated expression of a mouse steroid 21-hydroxylase/β-galactosidase transgene suggests centripetal migration of adrenocortical cells. *Mol Endocrinol* **10:** 585–598, 1996.
74. Tian Y, Balla T, Baukal AJ and Catt KJ, Growth responses to angiotensin II in bovine adrenal glomerulosa cells. *Am J Physiol* **268:** E135–E144, 1995.
75. McEwan PE, Lindop GB and Kenyon CJ, *In vivo* studies of the control of DNA synthesis in the rat adrenal cortex and medulla. *Endocr Res* **21:** 91–102, 1995.
76. Mitani F, Ogishima T, Miyamoto H and Ishimura Y, Localization of P450aldo and P45011 beta in normal and regenerating rat adrenal cortex. *Endocr Res* **21:** 413–423, 1995.
77. Bornstein SR, Ehrhart M, Scherbaum WA and Pfeiffer EF, Adrenocortical atrophy of hypophysectomized rats can be reduced by corticotropin-releasing hormone (CRH). *Cell Tissue Res* **260:** 161–166, 1990.
78. Tun RY, Smith MD, Lo SS, Rook GA, Lydyard P and Leslie RD, Antibodies to heat shock protein 65 kD in type 1 diabetes mellitus. *Diabet Med* **11:** 66–70, 1994.
79. Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horiuchi M, Pratt RE and Dzau VJ, The angiotensin II type 2 (AT_2) receptor antagonizes the growth effects of the AT_1 receptor: Gain-of-function study using gene transfer. *Proc Natl Acad Sci USA* **92:** 10663–10667, 1995.
80. Yamada T, Horiuchi M and Dzau VJ, Angiotensin II type 2 receptor mediates programmed cell death. *Proc Natl Acad Sci USA* **93:** 156–160, 1996.
81. Blauer KL, Poth M, Rogers WM and Bernton EW, Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. *Endocrinology* **129:** 3174–3179, 1991.
82. Bamberger CM and Chrousos GP, The glucocorticoid receptor and RU 486 in man. *Ann NY Acad Sci* **761:** 296–310, 1995.
83. Hayashi Y, Hiyoshi T, Takemura T, Kurashima C and Hirokawa K, Focal lymphocytic infiltration in the adrenal cortex of the elderly: Immunohistological analysis of infiltrating lymphocytes. *Clin Exp Immunol* **77:** 101–105, 1989.
84. Dhabhar FS, Miller AH, McEwen BS and Spencer RL, Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. *J Immunol* **157:** 1638–1644, 1996.
85. Dhein J, Walczak H, Baumler C, Debatin KM and Krammer PH, Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* **373:** 438–441, 1995.
86. Brunner T, Mogil RJ, LaFace D, Yoo NJ, Mahboubi A, Echeverri F, Martin SJ, Force WR, Lynch DH, Ware CF and Green DR, Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* **373:** 441–444, 1995.
87. Truman JP, Ericson ML, Choqueux-Seebold CJ, Charron DJ and Mooney NA, Lymphocyte programmed cell death is mediated via HLA class II DR. *Int Immunol* **6:** 887–896, 1994.
88. Yoshino T, Cao L, Nishiuchi R, Matsuo Y, Yamadori I, Kondo E, Teramoto N, Hayashi K, Takahashi K, Kamikawaji N and Akagi T, Ligation of HLA class II molecules promotes sensitivity to CD95 (Fas antigen, APO-1)-mediated apoptosis. *Eur J Immunol* **25:** 2190–2194, 1995.
89. Lynch DH, Biology of Fas. *Circ Shock* **44:** 63–66, 1994.
90. Alderson MR, Tough TW, Davis-Smith T, Braddy S, Falk B, Schooley KA, Goodwin RG, Smith CA, Ramsdell F and Lynch DH, Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* **181:** 71–77, 1995.
91. Newell MK, VanderWall J, Beard KS and Freed JH, Ligation of major histocompatibility complex class II molecules mediates apoptotic cell death in resting B lymphocytes. *Proc Natl Acad Sci USA* **90:** 10459–10463, 1993.
92. Arimilli S, Mumm JB and Nag B, Antigen-specific apoptosis in immortalized T cells by soluble MHC class II-peptide complexes. *Immunol Cell Biol* **74:** 96–104, 1996.
93. Weiss LM, Medeiros LJ and Vickery AL Jr, Pathologic features of prognostic significance in adrenocortical carcinoma. *Am J Surg Pathol* **13:** 202–206, 1989.
94. Oelkers W, Diagnostic puzzle of the adrenal "incidentaloma." *Eur J Endocrinol* **132:** 419–421, 1995.
95. Hirokawa K, Utsuyama M, Kasai M and Kurashima C, Aging and immunity. *Acta Pathol Jpn* **42:** 537–548, 1992.
96. Weksler ME, Immune senescence and adrenal steroids: Immune dysregulation and the action of dehydroepiandrosterone (DHEA) in old animals. *Eur J Clin Pharmacol* **45**(Suppl 1)**:** S21–S24, 1993.